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STUDIES ON *THELAZIA CALLIPAEDA* RAILLIET AND HENRY, 1910*

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Thelazia callipaeda was first described by Railliet and Henry (1910) from a single female specimen, obtained from the nictitating membrane of a dog from Rawal Pindi (Punjab). Later twenty other worms were apparently recovered from the same host. Evans and Rennie (1910) have also recorded the prevalence of this species of extra-ocular nematodes in dogs in Burma. The first record of the species in China is that of Stuckey (1917), who removed four specimens from the right conjunctival membrane of a Peking coolie and at the same time found similar worms in a foreign dog at Tungchow, a suburb of Peking. The same year Trimble removed worms which he believed to be the same species from the upper fornix of the right eye of a Fukienese farmer. In 1919 Fischer reported these worms from the eye of a native dog in Chungking, Szechuan. The worm is also believed to have been recovered from the stool of a Chinese patient after an anthelminthic (Barlow, 1921), but this seems very improbable. Recently the writer (1927), presenting results based on restudy of Stuckey's material in comparison with fresh specimens obtained from a foreign dog in Peking as well as from the right eye of a Peking laboratory rabbit, was able not only to confirm Leiper's view (1917) that the human and canine specimens from Chinese sources belonged to the species *Thelazia callipaeda,* but also that the worm from the rabbit belonged to this species. Within the past three years a considerable amount of data has been accumulated bearing on the biology, morphology, pathogenicity and geographical distribution of this nematode in China.

The adult worms are ectoparasitic on the conjunctival membrane, living in the conjunctival sac of the host, and wandering out from time to time across the eyeball, but returning sooner or later to their original location. They are bathed in mucous and lacrymal secretions which appear to be considerably increased in infected animals. In no case have the adult worms been found to enter the lacrymal duct or to burrow into the conjunctival tissue. Their food consists of epithelial cells and mucous secretions. Opportunity has been afforded to study the living

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worms in naturally acquired infections, *in vitro,* and after transplantation into laboratory animals. During the past eight years the infection has never been found in the Peking area in dogs living under native conditions, but dogs reared under foreign conditions from five separate localities in and around Peking have been found to harbor the parasites. The same hosts from three of these localities have been examined for three successive years, with the following results. The dogs have been found infected early in the fall and have been brought into the laboratory for study. After instillation of a 2 per cent cocaine solution into the conjunctival sac the worms become restive and can be readily picked out with eye forceps and removed to normal saline solution. In half an hour all of the worms in such an infection may be removed. With one exception reexamination of the host a week or two later has always proved negative. The following spring (April or May) the animals have also been found to be uninfected. During the summer a new infection is acquired, so that, on examination the following fall, worms are again found in approximately the same numbers. In one case the worms removed in October from both eyes of a dog that had been negative the previous spring were slightly immature, probably indicating that the period for complete development requires several weeks. Worms removed in November have always been mature. In no case has a natural infection in dogs been found to be unilateral. The two human cases on record involving the conjunctival sac (Stuckey, 1917, Trimble, 1917) also appear to have been contracted during the previous summer. The number of worms obtained in each new infection has varied from one to fifty. The relative number of males and females varies, but on the average the two sexes are equal in numbers.

The worms when removed to various physiological solutions remain alive for short periods of time, depending in part on the solution and in part on the temperature. The maximum period of vitality was found to obtain in physiological salt solution at 26° C., in which the worms were kept alive for ninety-six hours.

Tests have been made to determine the adaptability of the adult worms to the conjunctival membrane of various mammalian hosts. In each case several worms, consisting of an equal number of males and females, have been implanted into the conjunctival sac of the left eye, the right eye being left as a control. Altogether one dog, one monkey, four cats, four rabbits, one goat and one sheep have been utilized in this experiment. The results of the test (see accompanying table 1) indicate that the worms may be transplanted successfully to all of these animals except the goat and the sheep. All of the worms in the successful implantations were present up to one month, with the exception of one cat which had lost the infection. In the other cats the worms were lost between one and seven months. The monkey died on the thirty-second day after infection and at that time harbored all of the worms originally

implanted. The dog and the rabbits remained infected for more than a year, although in each animal some of the original worms had been lost. These data indicate that the goat and the sheep are entirely inappropriate hosts, that the cat is only a partially appropriate one, and that both the dog and the rabbit are highly suitable hosts. The experiment with the monkey was terminated too early to determine the degree of tolerance which the worms had for this animal. It it believed that the relatively large size of the "third eyelid" in both the dog and the rabbit, serving as a pocket for the worms, may be an important factor in assisting the parasites to retain their position on the surface of the eye. This belief is substantiated by the unsuccessful implantation of the worms in the sheep and goat, which lack a "third eyelid," and by the shorter period of tolerance of the infection in cats, in which the fold is

TABLE 1.—Showing the results of implantation of *Thelazia callipaeda* in the conjunctival sac of various mammals

Experimental Host	Examination for Presence of the Worms after						
	1 Day	2 Days	1 Week	1 Month	3 Months	6 Months	1 Year
Dog	+	+	+	+	+	+	+
Monkey	+	+	+	+	Host died on 32nd day when worms were all recovered		
Cat	+	+	+	+	—	—	—
Cat	+	+	+	+	+	+	Autopsied after six months; worms recovered
Cat	+	+	+	+	—	—	—
Cat	+	+	+	—	—	—	—
Rabbit	+	+	+	+	+	+	+
Rabbit	+	+	+	+	+	+	+
Rabbit	+	+	+	+	+	+	+
Rabbit	+	+	+	+	+	+	+
Goat	—	—	—	—	—	—	—
Sheep	—	—	—	—	—	—	—

only slightly developed. If this be true, neither monkeys nor man are optimum hosts for the worms.

During the period of the experiment, and subsequently, the right eye remained uninfected, indicating that there was no migration across the nasal bridge or via the lacrymal ducts from the infected eye to the control. Likewise, in most instances fewer worms were recovered at the end of the observational period than were originally implanted, indicating that they did not produce progeny *in situ*. In none of the other laboratory animals of the same species under observation in Peking have the worms been found during or subsequent to the experimental study.

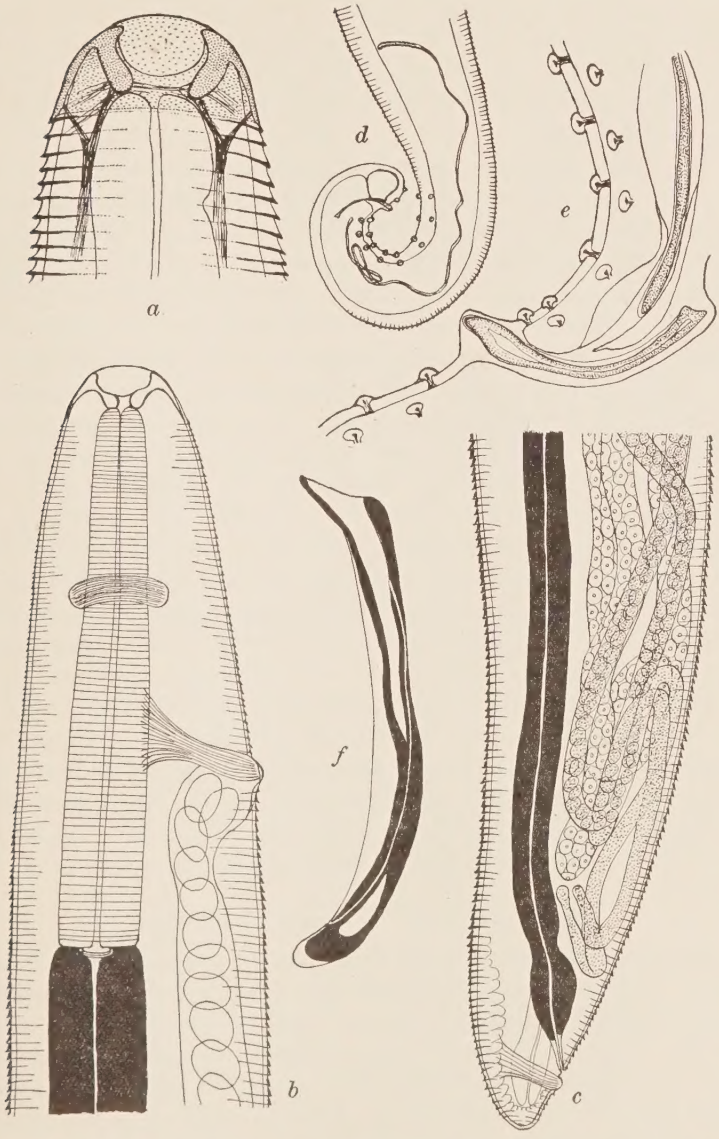
STRUCTURE OF THE ADULT WORM

Adult living specimens of *Thelazia callipaeda* are creamy or ivory-yellow in color, cylindrical in shape, and taper gradually towards both ends. Male worms range in size from 8.5 to 13 mm. in length by 0.275 to 0.75 mm. in greatest diameter, and female worms, from 12 to 16 mm. in length by 0.5 to 0.8 mm. in greatest diameter. The cuticula of the

entire body of the female, and that of the male to within a short distance of the posterior end, is transversely plaited or folded like that of Onchocerca so as to give the appearance of superficial segmentation. The edges of the folds are sharply serrated. The cephalic extremity is bluntly rounded (Text-fig. 1 *a*). The head is without lips but is provided with a capsule, continuous dorsally and discontinuously ventrally. The ventral plates of the capsule are club-shaped, are directed somewhat mesad, and extend posteriad to the anterior plane of the esophagus. The lateral plates, as seen in ventral view, form an acute angle with the ventral plates, but are in direct posterior continuation with the dorsal portion of the capsule. The dorsal plates, which appear in ventral view as short blunt processes, fuse with the laterals to form a capsular hood over the dorsum. Living worms show a perfect fusion of the two plates in the mid-dorsum. Railliet and Henry (1913) have observed six depressions on the anterior margin of the capsule, of which four (or less), submedian in position, are described as occupied by papillae. The writer has not seen these ornamentations either in living or in preserved material, but the possibility of such organs is indicated by the presence of six papillae in the first-stage larva. (See Text-fig. 2 *n*).

The short oral vestibule leads directly into the cylindrical esophagus (Text-fig. 1 *b*), which in female worms (of 16 mm. length) measures 0.86 mm. long by 85μ in cross section. The esophagus is anchored ventrally just behind its middle by a muscular attachment to the somatic wall. A circumesophageal nerve ring is situated about 0.3 mm. behind the anterior end of the esophagus. A small powerful spincter guards the opening of the esophagus into the mid-intestine. This latter portion of the digestive tract is a long rod-like organ, measuring about 80μ in cross section in male worms and 100μ in females. It is blackish in color, is less flexible than the other organs, and has no lateral anchorage along its entire length. Posteriorly it expands into a pyriform bulb (Text-fig. 1 *c*) before opening into the narrow cuticularized rectum. In both the males and females the mid-intestine is displaced toward the dorsal half of the worm by the reproductive organs.

The writer (1927) has previously described the accessory genital organs of the male worm. Study of a larger number of specimens requires the following additions. The number of pairs of preanal papillae (Text-fig. 1 *d, e*) varies from six to eight rather than being constantly six. The shorter of the two copulatory spicules (Tex-fig. 1 *f*) is seen in living specimens to be provided with thin curved lateral keels, forming a sleeve, which is closed at the outer tip and to which the solid portion of the spicule is fused. The tip of the spicule is also slightly twisted. The primary sexual organs of the male are described here for the first time. The tubular testis originates about 2 mm. from the anterior end of the worm in the mid-ventral aspect, where it is anchored. It proceeds about 0.25 mm. posteriad, coils once upon itself, then con-



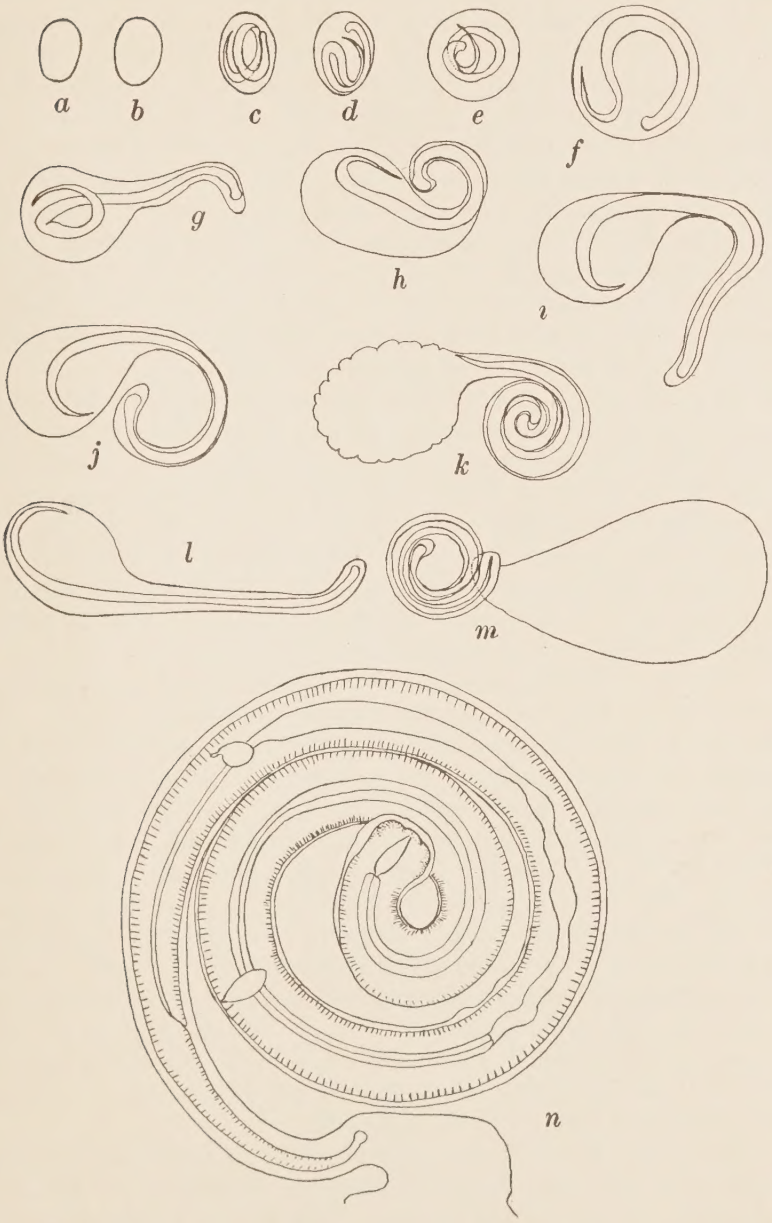
Text Figure 1.—*a*, anterior end of *Thelazia callipaeda*, ventral view, showing oral capsule with muscle insertions, coarsely striated cuticula and anterior extremity of esophagus, × 450; *b*, anterior end, female *T. callipaeda*, showing buccal opening, esophagus, anterior end of mid-intestine, circumesophageal nerve ring, vulva and anterior extremity of vagina; *c*, posterior end, showing mid-intestine, rectum, and coils of uteri, oviducts and ovaries, × 208; *d*, posterior end of male worm, with 8 pairs of preanal and 2 pairs of postanal papillae, and unequal copulatory spicules, × 67; *e*, detail of pericloacal region of male, showing short spicule and outer portion of long spicule in spicular pockets, and papillae adjacent to anal opening, × 227; *f*, short spicule, with alate margins, × 520.

tinues directly to the caudal extremity. It expands rapidly in diameter, reaching a maximum breadth of 200μ somewhat postequatorially, then gradually decreases in size, to be continued into the ejaculatory duct which opens into the cloaca. No distinct vas deferens or vesicula seminalis has been seen.

The female worm has a prominent vulva on the mid-ventrum (Text-fig. 1 *b*), some 0.55 to 0.6 mm. behind the cephalic end and about 0.26 mm. in front of the posterior termination of the esophagus. The vagina is directed posteriad for a distance of 1.40 mm. (i.e. 2.0 mm. behind the anterior end of the worm), where it expands into a bulbous ovejector. This latter organ leads posteriorly into the uterus, which consists of a single stem for a distance of 0.7 mm. At this point (2.7 mm. behind the anterior end) it bifurcates, the two arms paralleling one another as they continue posteriad. About 1.0 mm. from the caudal extremity (Text-fig. 1 *c*) the two uteri turn abruptly forward and proceed anteriad for about 5 mm., then again coil back and forth for the same distance through one and one-half complete loops, finally becoming reduced in size and continuing as oviducts. These oviducts proceed to within 10 mm. of the caudal end, loop forward a short distance, and again coil posteriad, this time as the ovarian tubules. The innermost blind ends of the tubules are anchored to the somatic wall slightly anterior to the posteriormost portion of the tubules. Separate egg cells are first observed in the oviducts. In the inner loops of the uteri they are closely packed together. In the outer portion of the uteri and in the vagina eggs from early cleavage stages to fully formed embryos coiled within the egg membrane are successively found. In contrast to the observation of Railliet and Henry (1913) the writer has found the number of these embryonated eggs to be very great.

THE LARVA OF THELAZIA CALLIPAEDA

The eggs of *T. callipaeda* are fully embryonated at the time of oviposition. They are oval in contour, with a transparent shell membrane which is slightly greenish in color. Eggs *in utero* (Text-fig. 2 *a, b*) measure 54 to 60μ in length by 34 to 37μ in transverse diameter. The larvae are tightly coiled within their shells and the eggs are closely packed together within the uterus. The movement of the larvae within the egg-shells together with the contraction of the vagina appear to be responsible for egg-deposition. When placed in isotonic saline solution living females oviposit at irregular intervals. Very soon after the eggs are laid *in vitro* the enveloping membrane begins to swell, at first maintaining the oval contour (Text-fig. 2 *c, d*) but later becoming spherical in outline (Text-fig. 2 *e, f*), and measuring up to 87.5μ in diameter. Within this spherical membrane the larva moves about quite freely, coiling and uncoiling at regular intervals, but always returning to the condition of a watch-spring coil. Shortly after this period (an hour or two,



Text Figure 2.—Successive stages in the development of the egg envelope of *T. callipaeda*. *a-m*, × 187; *n*, × 750.

depending on the activity of the larva) the head end of the larva begins to produce a finger-like evagination on one side of the egg-membrane (Text-fig. 2 *g*), the process being extended until the entire larva has provided for itself a closely fitting sheath, opening at the caudal end of the worm into the enlarged bladder which has developed from the "ballooning" of the greater part of the original egg-membrane (Text-fig. 2 *h-m*). Frequently the tail portion of the larva projects back into the bladder, but as a rule the entire larva remains within the finger-like projection, which becomes tightly coiled in one plane to conform to the coiling of the larva within. Examination of the conjunctival secretion of infected animals usually reveals several such larvae with their enveloping membranes in process of evolution from the small oval egg structure to the bladder type with its coiled appendage.

After this modification of its contour the bladder floats on the surface of isotonic or hypotonic liquids, with the coiled larva suspended underneath. This appears to be the normal condition of the larval membrane at this period of development, since it is commonly present in natural secretions of the eye as well as in isotonic saline solution, and has even been observed *in utero* in dying females. Within the membrane-sheath the larva now becomes quiescent. Although the sheath is extremely thin it is resistant to desiccation for several days as well as to dog's fresh gastric and intestinal juices.

The mature first-stage larva (Text-fig. 2 *n*) has a total length measurement of about 400μ and a greatest transverse diameter of 13.3μ. The head end is bluntly rounded and is apparently provided with six inconspicuous circumoral papillae. The posterior end of the larva begins to decrease in diameter just behind the anal pore, becoming narrower and narrower caudalwards but terminating in an enlarged caudal process. Within the mouth there is a small buccal vestibule, but no capsule is present. The esophagus extends to the equatorial plane. The anal pore lies 50μ in front of the caudal extremity. The excretory bladder is a lenticular vesicle 150μ behind the cephalic extremity. A nuclear mass, possibly the genital primordium, is located about 50μ in front of the anal pore. The entire body is delicately striated. Occasionally the larvae in isotonic saline solution effect an exit from the enveloping sheath and wriggle about for a short time in the medium. Their vitality, however, soon decreases and in a few hours they die.

The method by which the larvae are able to complete their development and as adults take up their residence in the conjunctival sac of an appropriate mammal is still obscure. Various experiments planned to test direct and insect transmission have thus far been fruitless. Larvae, secured directly from conjunctival secretion as well as from isotonic saline solution in which female worms had been ovipositing, when instilled in the conjunctival sac of uninfected dogs and rabbits, have never

FIG. 1—SECTION THROUGH SKIN IN VICINITY OF SEBACEOUS GLAND, SHOWING FRAGMENTS OF THELAZIA CALLIPAEDA LARVAE IN DUCT. X 95.

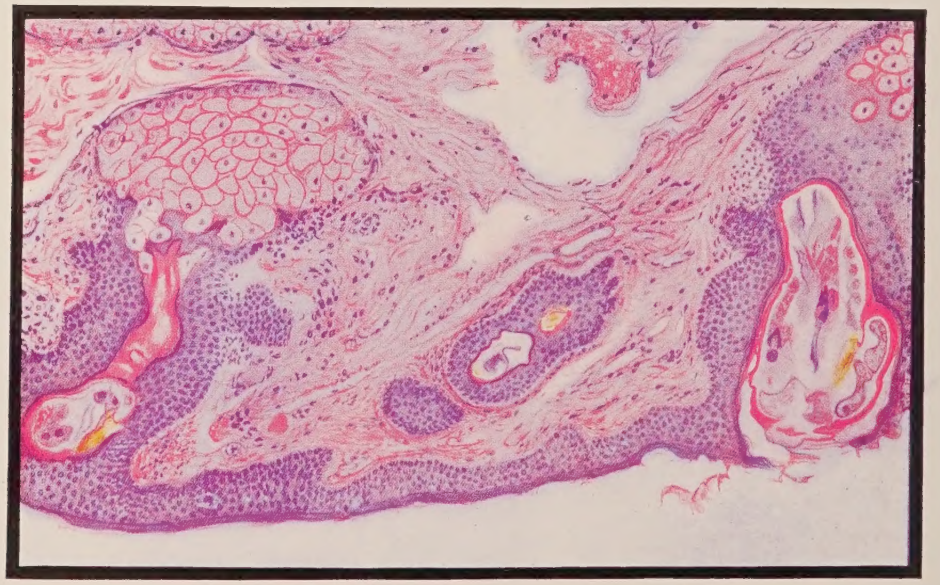


FIG. 2—ADJACENT AREA FROM SAME SECTION, FRAGMENTS OF THELAZIA CALLIPAEDA LARVAE (LEFT), IN DUCT LEADING INTO SEBACEOUS GLAND, SINGLE LARVA (CENTER) IN DUCT, AND NEST OF LARVAE (RIGHT) IN EPITHELIAL POCKET. X 95.

FIG. 3—DETAIL OF THREE THELAZIA CALLIPAEDA LARVAE IN SECTION OF EPITHELIAL POCKET FROM SAME CASE AS FIGS. 1 AND 2. THE ANTERIOR ENDS OF THE WORMS ARE AT THE INNER END OF THE POCKET. THE BUCCAL CAPSULE IS SHOWN IN THE LOWERMOST ONE. X 800.

FIG. 4—DETAIL THROUGH ANOTHER EPITHELIAL FOLD, STRONGLY CORNIFIED, SHOWING PORTIONS OF THELAZIA CALLIPAEDA LARVAE IN SECTION. THEIR HEADS ARE DIRECTED INWARD. THE INTEGUMENT OF THE LARVA SHOWS THE CHARACTERISTIC SERRATED PSEUDO-SEGMENTATION. X 800

developed beyond the stages observed *in vitro*. Even when the conjunctival membrane has been previously scarified the results are the same. As a test of mechanical transmission uninfected rabbits have been placed in a cage with infected animals or with female worms ovipositing *in vitro*. Various species of filth flies, the dog fly, *Hippobosca francilloni,* and the cockroach, *Blatella germanica,* have been introduced. The uninfected animals have not only remained negative but the insects, which have been observed feeding on the liquid containing the larvae, have also remained consistently uninfected, although larvae have been found entangled in their mouth parts. The possibility that certain insects, capable of serving as intermediate hosts, and developing in endemic foci only during the summer months when the canine hosts appear to acquire their infection, has not been ruled out. Certain it is that the larvae deposited by the female worms are capable of development *in vitro* only to a certain point, and that they are provided with a resistant enveloping membrane which protects them from desiccation and, because of its bladder float, also prevents them from remaining immersed in liquids. This suggests the possibility of a period of inactivity of several days or even weeks, during which the ensheathed larvae, either in a dry or moist medium, are viable and may await passive transmission to the next host.

One human case, recently reported by Howard (1927), in which larvae of *Thelazia callipaeda,* in an advanced stage of development, have been found in a papilloma of the skin of the lower right eyelid near the internal canthus, may possibly have a bearing on the early stage of the mature infective larvae of this species present in mammalian infections. The patient from whom the papilloma was excised gave a history of having fondled a pet dog which had an irritation of both eyes, although "eye worms" were not actually observed in the dog. There was no evidence that the patient himself had ever been parasitized by adult Thelaziae. The papilloma, which had been present for many years, developed a persistent itching several months previous to Dr. Howard's examination of the case. The patient was conscious of having frequently rubbed the wart during the period when the dog was being petted. This scratching of the lesion resulted in the development of an indurated scab-like encrustation around it.

Microscopic slides of the excised papilloma, showing the worms in position in the tissue, were referred to the present writer for diagnosis. The nematodes (colored plates, figures 1-4) were directed head-inward into pockets produced by the infolding of the epithelium, or had migrated into the ducts of the sebaceous glands. In the former case the epithelial folds were strongly cornified and the tissue just beneath the epithelium was characterized by a marked lymphocytic infiltration. Two or more worms were found in each focus. The worms measured about 0.5 to 0.6 mm. in length by 40 to 50μ in breadth, and had a serrated pseudoseg-

mentation from anterior to posterior ends identical with that of adult *Thelazia callipaeda*. The buccal capsule was also of the same type as that described by the writer for adult worms of this species. Attached to various parts of the buccal capsule were muscle elements which apparently served to regulate the size of the oral opening. A muscular esophagus was found in the anterior portion of the worms, while in the more distal region the intestinal tract was filled with various cellular elements from the host. The posterior end of the female worms was bluntly rounded; in the sections examined the more attenuate posterior end of the males was cut off in the longitudinal plane. There was a group of embryonic cells in the region of the vulva of the female worms and another group further caudad, indicating that the genital primordia were still undifferentiated. It is evident that these larvae were much further developed than those maturing in the embryonic egg envelope. They were not only somewhat longer and much broader but possessed cuticular and buccal elements similar to those of the adult worms. It seems clear that the lodgement of these larvae in a papilloma of the skin of man was accidental. The manner in which the larvae reached this location is unknown and their unnatural habitat is probably of no great value in the elucidation of the natural life cycle of the organism; however, information is provided concerning an advanced larval stage of the worm. It is likewise important to note that the larvae in this human case were essentially ectoparasitic in their habitat, as are the adult worms. It is reasonable to believe that the interval between the first larval stage within the embryonic envelope and the mature larvae, which strikingly resembles the parent worm, is passed as an extra-mammalian part of the life cycle. Even though experimental evidence is thusfar negative, an intermediate insect host is probably indicated, particularly in view of the fact that the closely related "eye worm" of Australian poultry, *Oxyspirura parvovum* Sweet, 1910 and Manson's "eye worm" of chickens, *O. mansoni* (Cobbold, 1879) are both known to involve insects as intermediate hosts (Fielding, 1926; Nishigori, *fide* Professor S. Yokogawa).

PATHOGENICITY AND SYMPTOMATOLOGY IN ANIMALS

The adult *Thelazia callipaeda* commonly lives in the inner canthus of the eye, making excursions from time to time over the surface of the eyeball, the movement consisting of a graceful serpentine glide. Sooner or later the worm returns to its primary location. In ordinary cases of infection, where secondary invaders have not secured a foothold, the conjunctiva is very little inflamed and the eyeball is not seriously affected. In animals having recurrent infections from year to year a cloudiness gradually develops over the eyeball, observed first on the inner aspect and progressing outward across the eyeball. The writer has studied this opacification in five naturally infected dogs over a

period of three years. In each case the opacity, which was at first barely noticeable and caused no inconvenience to the dog, has spread outward over the organ, at the same time becoming more intense, so as to reduce the dog's vision to a marked degree. This cloudiness, which involves only the superficial layers of the eyeball, is apparently the result of repeated abrasion of the epithelium of the organ by the serrated cuticula of the worm, causing microscopic scarification, with resultant scar-tissue formation.

The irritation produced by the presence of the worms moving over the conjunctival membrane, particularly in the vicinity of the inner canthus, gives rise to a general nervous reaction on the part of the host. The human case reported by Trimble (1917) complained of pain in the affected eye covering a period of three months, with ectropion of the lower lid which allowed a constant flow of tears to run down the face. This was believed to be due in part to a decrease in the muscle tonus of the parasitized area. The symptoms disappeared with the removal of the parasites.

In all parasitized animals which the writer has observed there is an excess of lacrymal secretion. The pocket produced by the "third eyelid" in the dog and the rabbit becomes bulged out, thus accommodating the worm mass and the excess of secretion, so that ectropion of the eyelids of these animals is uncommon. The lacrymal and mucous secretions also tend to be accommodated in the enlarged conjunctival sac, the secretions drying in masses and at times plugging the opening of the lacrymal duct. In cats and in the one monkey in which the adult worms were experimentally implanted excessive flow of secretions out of the infected eyes was observed but no ectropion was noticeable.

Present information limits the distribution of *Thelazia callipaeda* in the Far East to China. It has not been reported from Japan or Korea and is unknown in Formosa (*fide* Yokogawa). In China the worm has been found in dogs in North China (Peking area) and the Upper Yangtze Valley (Chungking and Chengtu, Szechuan). Human cases are known from Peking (Stuckey, 1917), Fukien Province (Trimble, 1917) and Chengtu, Szechuan (Howard, 1927). The single record of this species as a natural infection in the rabbit is from Peking (Faust, 1927).

SUMMARY

1. The biology, morphology, pathogenicity and geographical distribution of *Thelazia callipaeda,* from the conjunctival membrane of various mammals, was studied.

2. Natural infection in dogs appears to be acquired during the summer months.

3. Dog and rabbit are optimum hosts, cat and monkey are only semi-suitable ones, and goat and sheep are entirely unsuitable. These data

correspond to the development of the nictitating membrane of these animals.

4. In appropriate hosts the number of worms becomes reduced over a period of months; the worms in control animals do not increase in numbers or migrate from an infected to a non-infected member.

5. The structure of the adult worm and the development of the first-stage larva are described in detail.

6. A mature larval stage in a papilloma of the lower eyelid of a human host is structurally similar to the adult worm.

7. Attempts to elucidate the life cycle were unsuccessful but an arthropod vector is not excluded.

8. Continuous infection in dogs develops an opacity of the surface of the eyeball and an excess of mucous and lacrymal secretions in all hosts but ectropion of the lower lid, described for a human case, has not been seen in other hosts.

9. Infection with *Thelazia callipaeda* in the Far East is apparently confined to China, where cases have been reported from Peking, West China and Fukien Province.

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AN EXPERIMENTAL STUDY OF SOME EFFECTS OF *CYSTICERCUS FASCIOLARIS* RUD. ON THE WHITE RAT*

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Cysticercus fasciolaris is the larval stage of *Taenia taeniaeformis,* adult in the intestine of the cat. When encysted in the liver of the intermediate host, the mouse or the rat, *C. fasciolaris* may affect its host in at least two ways: first, by mechanical obstruction of the liver tissue, by blocking the ducts or capillaries; and second, by secreting substances which, on passing through the cyst wall, have some harmful consequence. Descriptions in the literature of the gross effects of *C. fasciolaris* on the mouse and rat are conflicting; no experiments to determine less obvious effects have been made, other than those concerning the production of sarcoma.

The present study has to do chiefly with the observation of changes in the blood of the rat following infestation; but other aspects of the host-parasite relationship are also dealt with. The effects of parasites on the blood picture have usually been studied in naturally infested animals concerning whose previous history nothing is known, or in laboratory animals into which worm products or extracts have been injected. There are few studies in which progressive changes in the blood have been followed in a natural host, experimentally infested.

Cysticercus fasciolaris is admirably suited to a study of the kind to be described in this paper. The adult, *T. taeniaeformis,* can be obtained from the cat, and the oncospheres of the mature proglottids are easily fed to the rat. The developmental stages are well known; the most recent study is that of Bullock and Curtis (1924). After the larvae reach the capillaries of the liver by way of the portal system they remain there, growing within the individual cysts which the liver secretes around them. This makes them a much more certain factor to deal with than are intestinal worms, which may be expelled from the body or whose toxic products, if formed, may be absorbed by the intestinal contents and carried out of the body. Furthermore, there is no multiplication of these cysticerci in the body of the host as is the case of parasitic protozoa

* All of the erythrocyte and differential blood counts were made by the junior author; most of the other data represent a joint contribution.

in the intestine or blood stream, or, for example, of Trichinella among the metazoa; and consequently the effects of a definite number of worms can be studied. As the rat is a normal host of *C. fasciolaris* the reactions to the parasite are such as might occur naturally. In this connection it is felt that in too many experiments products or extracts of worms have been injected into animals which do not serve as hosts to the particular parasite and that the conclusions drawn from such experiments are of doubtful value; attention has been called to this fact by Parisot and Simonin (1920:739).

Another advantage offered by *C. fasciolaris,* as well as by *E. granulosus, C. tenuicollis* and other cysticerci, lies in the fact that the cyst fluid is a natural one, in contrast to the various extracts of worms which have been injected into animals in the study of parasite toxins and of anaphylactic phenomena. Furthermore, the rat is a small laboratory animal and a quantity of cysts may be available at any time, although the volume of cyst fluid which may be obtained is small in comparison to that which may be had from *C. tenuicollis* from slaughterhouse sheep.

It is true that the number of cysts of *C. fasciolaris* experimentally introduced into the liver in the present experiments is often far in excess of the number generally found in nature. In wild rats and mice it is common to find only a single cyst, or at the most a small number. Some exceptional cases are recorded; Smith (1908) mentions a muskrat liver containing several hundred cysts and being enormously enlarged in consequence, and Hopwood (1914) published a brief note calling attention to an infestation of over 250 cysts in a wild rat. The overfeeding of some of the animals of the present experiments was done deliberately in order to determine whether the excessively large dose would increase or decrease the reaction of the rat.

The hosts used in these experiments were kindly furnished by Dr. F. B. Hanson of Washington University; they were 14-16th generation rats of a highly inbred stock, which was started in 1920 from one pair of Wistar Institute rats. As often as was possible, litter mates, or at least rats of the same age, sex, and approximate weight were used in each experiment; this reduced individual variation to a minimum. In this stock of laboratory bred animals the presence of endoparasites and ectoparasites is rare. This is an advantage over the use of slaughter house animals where one has no way of knowing the previous infestation of parasites which have died and been thrown off; and it is known that in some cases an eosinophilia may persist for a long time after the removal of the parasites. Nor in the case of slaughter house animals can it be certain which of the many parasites in the viscera and elsewhere may be causing the reaction.

The rats, ordinarily fed in the evening, were caged individually over night without feeding, and in the morning were given food containing the oncospheres. These were obtained by teasing apart the terminal proglottids of live worms and suspending the oncospheres in physiological salt solution. A certain number of drops of the suspension was then placed in a depression in a small ball of moist, cooked mash, which absorbed the water. Although only fresh oncospheres were used it was found that they are somewhat resistant to dessication. Equal amounts of an oncosphere suspension were put on pieces of bread (15 by 15 by 5 mm.) which were stored in a covered petri dish until used. Each of a series of rats was fed one piece of this bread containing oncospheres at intervals up to three days. The resulting infestations were approximately uniform (about 400 cysts) for all feedings up to 48 hours, when the bread was quite dry; a single feeding at the end of three days resulted in only ten cysts.

The blood for the blood counts and smears was obtained from the tail except where otherwise stated; the end of the tail was cut off and the first drop was never used. Blood counts were made with the Levy-Hausser, double Neubauer counting chambers. Toisson's diluting fluid was used for erythrocytes and 3% glacial acetic acid for leucocytes. For erythrocytes the average of one hundred small squares was taken and for the leucocytes the average of five large ones. For the differential counts the smear was made by spreading the drop on a slide with a cigarette paper; Wright's blood stain was used. Five hundred cells were counted in the determination of the degree of eosinophilia.

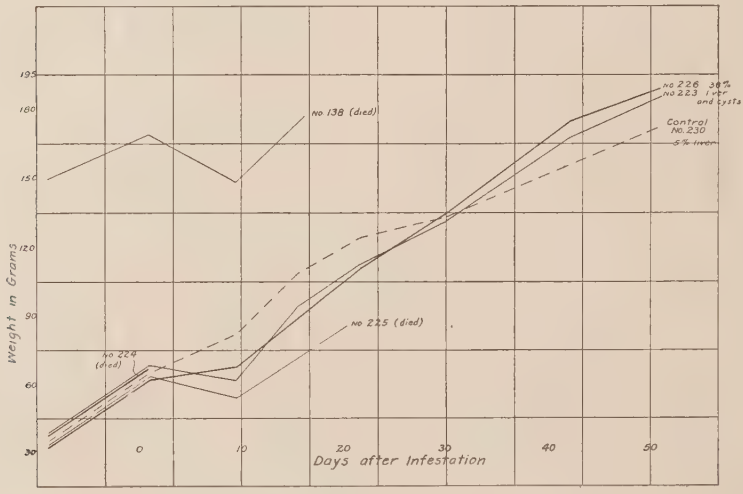
Every attempt was made to keep conditions uniform. The rats were fed at approximately the same time each evening and the blood counts made in the morning, in order to eliminate any variations that might occur in the blood due to nutrition.

RESULTS OBTAINED

The effects of *Cysticercus fasciolaris* on the health of the mouse, the other common host of this cestode, were first discussed by Raum (1883), who stated that a heavy infestation caused the mouse to become emaciated, and that death followed due to chronic intoxication. Vogel (1888) on the contrary maintained that the presence of numerous cysts (number not stated) did not seem to have any particularly harmful effect upon the health of the mouse. Judging from these results, and from the work of others, including that of Koninski and of Bartels, it appears that a considerable number of cysts must be present to affect the health of the host, and that the differences reported are due to differences in the degree of infestation.

In the present paper infestations of less than 25 cysts are considered light; between 25 and 100, medium; over 100, heavy; and the presence of several thousand cysts is considered a very heavy infestation. If the number of oncospheres fed is small, no general effect on the rat is observable. But if the dose given is a very heavy one, about a week or ten days later there is a drop in weight, accompanied by a general listlessness, and the hair becomes unkempt and the eyes inflamed. As the cysts grow they add considerable bulk to the liver, so that in extreme cases the abdomen protrudes greatly (Fig. 2).

The effect on the total body weight of animals having 500-600 cysts in the liver is shown in Text-figure 1, which is typical of rats with a

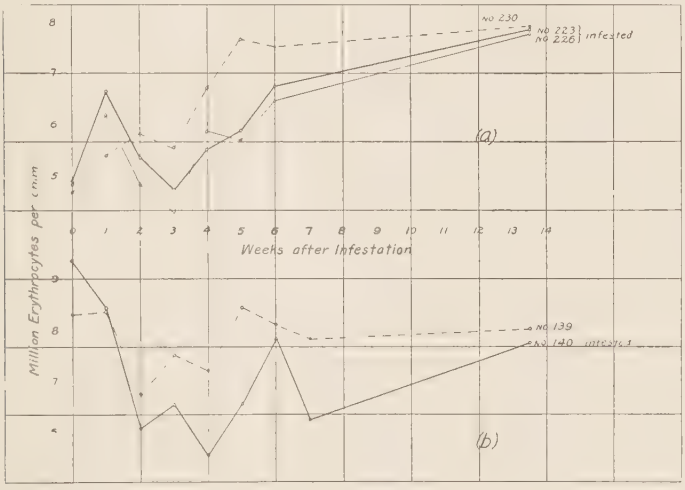


Text-fig. 1.—Graphs showing the changes in total body weight following infestation with *C. fasciolaris*. Five of the rats were brothers, of nearly the same body weight, and 60 days old when infested. Three of the six died as the result of the very heavy infestation. While the weights of rats Nos. 223 and 226 were not much greater than that of the control (No. 230), in the former the liver with the contained cysts represented 38% of the total weight while the liver of the control rat was only 5% of the body weight.

heavy or very heavy infestation. The graph varies from the normal less when the infestation is lighter; and there is no observable difference when the number of cysts is approximately that found in nature in the wild rat. It will be noted that the weight drops during the first week after infestation, followed by a slow rise, and that after a month the graph for the heavily infested rat crosses that of the control and then remains above it. This is due to the increased weight of the liver with its cysts, which in extreme cases becomes over one-half the weight of the entire animal. In these cases the rat becomes so emaciated that the backbone protrudes and the face has a characteristic sharp-pointed look.

In spite of these very marked effects, death is surprisingly uncommon, and when it does occur, it is as often in a rat with a medium-heavy infestation as in one with the liver almost completely filled with cysts. Occasionally animals with heavily infested livers had litters normal as to number and weight of the young.

That anemia often accompanies infestation with parasitic worms has been shown by many investigators. Injection of certain products and extracts of worms is also known to produce an anemia in some animals. Weinberg (1912: 1022) states that erythrocytes under the influence of toxic products become unfit for their physiological function and are rapidly destroyed by the phagocytes. Simonin (1920: 217) found that



Text Figure 2.—Graphs showing changes in erythrocyte counts of infested and control rats for a period of about 14 weeks after infestation. The graphs for variation in weight of rats Nos. 223, 226 and 230 were shown in text-fig. 1.

hydatid fluid has a weak toxicity, because repeated injections into rabbits and guinea-pigs failed to change appreciably the erythrocyte count.

In the present investigation erythrocyte counts of uninfested, control rats and experimentally infested rats were made just previous to the infestation and at varying intervals afterward. In one experiment three 60 day old male rats, litter mates and of approximately the same weight, were used. One was retained as a control (No. 230) and two (Nos. 223, 226) were infested with about 600 cysts each. Erythrocyte counts were taken at intervals of a week for six weeks and then again at three months from the time of infestation (Text-fig. 2 *a*).

The three rats had practically the same erythrocyte count, 4,800,000, at the beginning of the experiment; although the number varied from week to week, at each point beyond the first week the counts of the two

infested animals were lower than that of the control; but at the end of three months the counts were again practically the same for all three animals.

Similar results were obtained with two other rats (Text-fig. 2 *b*); these were females, litter mates. The rat experimentally infested was found to have about 300 cysts in the liver when killed. As in the first experiment, the erythrocyte count of the infested animal, after the first week, was constantly below that of the control.

Blood counts were made every two or three days on two rats having about 100 cysts each; each showed a decided decrease in the number of erythrocytes. Unfortunately both animals were killed under anesthesia, one thirteen days and the other nineteen days after infestation.

Since the parasite is encysted in the liver, it cannot ingest blood corpuscles or produce hemorrhages. The anemia may be due to a destruction of erythrocytes by toxic products of the worm, which have dialyzed through the cyst wall, or to the action of these substances on the hematopoietic organs; or it may be due to malnutrition caused by interference with the function of the liver. But on the other hand the decrease in the number of erythrocytes may be only apparent, due to an increased water content of the blood. This was not investigated.

In order to determine whether the erythrocytes of infested and control rats differ in their resistance to hemolysis by hypotonic salt solution, two fragility tests were made. In each case one infested and one control rat were used; these were litter mates, of the same sex and approximate weight. The technique of Griffin and Sanford was used. Blood was obtained from the portal vein, and a drop was added to each test tube in a series of twelve, containing sodium chloride (c.p.) solutions ranging from 0.28% to 0.50%. In each test one such series of tubes was set up with the blood of the rat which was heavily infested and compared with the control series which contained blood from the uninfested rat. The tubes were allowed to stand at room temperature and were read after two hours.

The most noticeable difference between the two series, in both tests, was that at all dilutions the contents of the control tubes were of a brighter red color. In both tests it was found that in the tubes containing the blood of the control rat hemolysis began at about 0.48% and was complete at about 0.40%, whereas in the tubes containing the infested rat's blood hemolysis began at about 0.44% and was complete only at 0.34%. This indicates a slightly increased resistance in the erythrocytes of the infested rats.

Correlating this slightly increased resistance with the decreased number of red cells, it might be supposed that *C. fasciolaris* secretes some substance which hemolyzes the weaker erythrocytes, and that those

remaining are therefore more resistant to hemolysis. This, however, is contrary to the opinion of Simonin (1920: 224), who stated that injection of hydatid liquid, or other products of worms, renders the erythrocytes more fragile and less resistant, even if it does not actually hemolyze them. On the other hand, if the anemia is more apparent than real, being caused by an increased water content of the serum, that in itself might explain the increased resistance, as it is known that the resistance of the red blood cells of the rat varies directly with the water content of the serum.

The effect of extracts of worms and of worm products on the rate of coagulation of whole blood *in vitro,* and the very much delayed coagulation which results from intravenous injections have been studied by several investigators, particularly for nematodes. Giusti and Hug (1923) have recently reported the noncoagulability of dog's blood following intravenous injection of hydatid fluid. In the present investigation Sabrazes' method for the determination of the rate of coagulation was used in the examination of the blood of thirty rats, fifteen infested and fifteen control animals. All factors were kept as uniform as possible; the tests were made during the same two hour interval of each day, and blood was always taken from the tip of the tail, after the first drop had been discarded. Instead of holding the capillary tube in the hand it was placed on a piece of paper immediately after being filled, and was only picked up at the times of examination. The room temperature varied from 19-21°C.

The mean of the readings taken on infested rats, under the above conditions, was 106 ± 2.5 seconds, while that for the same number of control animals was 96.7 ± 3.9 seconds; the difference, which is 2.03 times the probable error of the difference, is probably not significant in view of the relatively small number of tests and because of the experimental error in determining the exact moment at which coagulation took place.

Determinations of the amount of hemoglobin in the blood, by means of the Newcomer hemoglobinometer technique, were made on twenty adult infested and control rats; on some of these as many as four determinations were made, on different days. The results show no correlation whatever between the percentage of hemoglobin and the degree of infestation. Some of the lightly infested animals consistently had abnormally low percentages, between 71% and 75% hemoglobin, while some of those with heavy infestations showed readings of more than 100% hemoglobin. The range of variation in all rats examined was from 71% to 111%.

Bryce, Kellaway and Williams (1924) found that some hydatid fluids have a distinct hemolytic effect on sheep corpuscles and that this

property of the fluid was not altered by heating for one hour at 55° C. In view of the fact that low erythrocyte counts were found in the present work in rats infested with *C. fasciolaris,* the cyst fluid was examined for the presence of a hemolytic substance which, having dialyzed through the cyst wall, might destroy the red blood cells.

Repeated tests failed to show that fresh cyst fluid possesses any hemolytic effect on the washed red blood cells of infested or control rats, at refrigerator, room, or body temperatures. The results of comparative tests, using fresh fluids as well as various kinds of extracts of fresh and dried cysts of different stages of development and of cysticerci from old cysts, will be published subsequently. There is some evidence from this latter work that the erythrocytes from infested rats are more resistant to the action of cyst extracts which have hemolytic properties than are the erythrocytes from normal rats; if similar results are had from further experiments they will support the fragility test results.

In order to point out clearly the character of the present work on *C. fasciolaris,* it is necessary to discuss briefly what has previously been done, especially with larval cestode material, on eosinophilia associated with or due to parasites; the most recent extensive surveys are those of Weinberg and Leger, and Schwarz (1914).

Studies on general eosinophilia in its relation to worm parasites fall into at least four classes, of which the first and the third are by far the largest. (1) Eosinophilia observed in man; the data from the numerous studies on Echinococcus lead one to suspect that there might be no constancy in the occurrence or degree of eosinophilia due to *C. fasciolaris.* Weinberg and Leger concluded that the degree of eosinophilia in human Echinococcus infestation is most variable and has no apparent relation to the volume, size, or necessarily to the age of hydatid cysts. They believe that the determining factor is the amount of fluid which passes through the cyst membrane. (2) Observations on eosinophilia in slaughter house and other animals, concerning whose infestation nothing is known previous to autopsy. (3) Eosinophilia following the injection of fluids or extracts of parasites into laboratory animals.

(4) Studies on animals which have been experimentally infested with a particular species of parasite. The ideal method consists in experimentally infesting laboratory-bred, parasite-free animals of the same species as the natural host. This was done in the present study on *C. fasciolaris,* and so far as the authors are aware it is the first investigation in which larval cestodes have been experimentally introduced into uninfested animals and the variations in the number of eosinophile cells regularly followed.

Experiment 1. In order to ascertain in a preliminary fashion, whether an eosinophilia may result from infestation with *C. fasciolaris,* differential blood counts were made on a group of eleven infested and five uninfested rats picked at random from the general stock. From one to five counts were made on each rat. The average percentage of eosinophile cells for the infested rats was 6.1, while that for the normal animals was 1.9. This would seem to indicate that an eosinophilia existed; but while the number of eosinophile cells in the normal rats was below 4% in all but one instance (4.7%) and in the infested rats rose as high as 25% in one count and 10% in two other cases, there were a few infested rats which had an eosinophile cell count lower than that of the average for the normal rats. Hence it was necessary to have a much larger number of observations; and, furthermore, it was desired to trace the development of the eosinophilia by examinations at intervals after infestation.

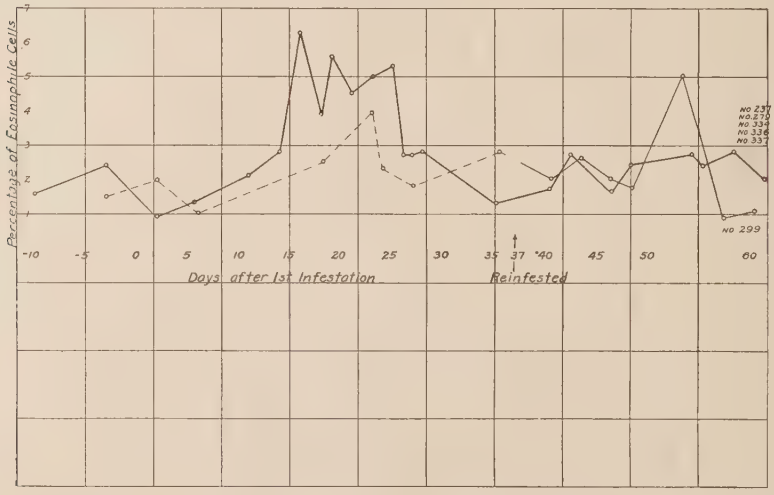
Experiment 2. The six rats used were all females, from two litters born on the same day; the range of their body weight was between 131 and 149 grams, and they were 105 days old at the time of infestation. They were fed equal doses of oncospheres; but on autopsy nine weeks later numbers of cysts, five millimeters in diameter, varying from none to 75 were found. Therefore, in no case was there a heavy infestation. Differential counts, made at least once for each rat before infestation, were continued at intervals of from one to eleven days, for a total of 37 days after feeding the oncospheres (text-fig. 3). The average percentage of eosinophile cells for five of the six rats is plotted, and the graph for the sixth one shown below it; this rat (No. 299) was fed the same-sized dose given the others, but there were no large cysts present upon autopsy.

Inspection of the graph shows that it rises rapidly between the 14th and 16th days after infestation, remains at a generally high level, and then drops off after the 25th day and remains low. Apparently rat No. 299 also reacted to the invasion of the oncospheres, although none developed in the liver. This high percentage of eosinophile cells, maintained from the 16th to the 25th day, may be an expression of the resistance of the host to the invading parasite, for it begins at the same time and continues beyond the time of the peak of cell proliferation around the oncospheres which Bullock and Curtis (1924: 451) found to occur between the 15th and 20th days and then to subside gradually.

Partly in order to see whether this rise in number of eosinophile cells would be repeated following a second feeding of oncospheres, on the 37th day after the first infestation all six rats were again given equal numbers of oncospheres in their food. On autopsy made four weeks later (nine weeks after the first infestation) from six to twelve small

cysts, 1 mm. in diameter, were found in the liver, in addition to the 5 mm. ones from the first feeding. Inspection of the graph for the five rats shows that there is no second eosinophilia comparable to that following the first infestation.

Rat No. 299, with twelve 1 mm. cysts in the liver on autopsy, shows a high percentage of eosinophile cells fifteen days after the second feeding; but no conclusion can be reached from a single case, especially as a similar, but not so great, increase in percentage of eosinophile cells followed the first, unsuccessful feeding of oncospheres. It is not clear why only the second infestation was successful.

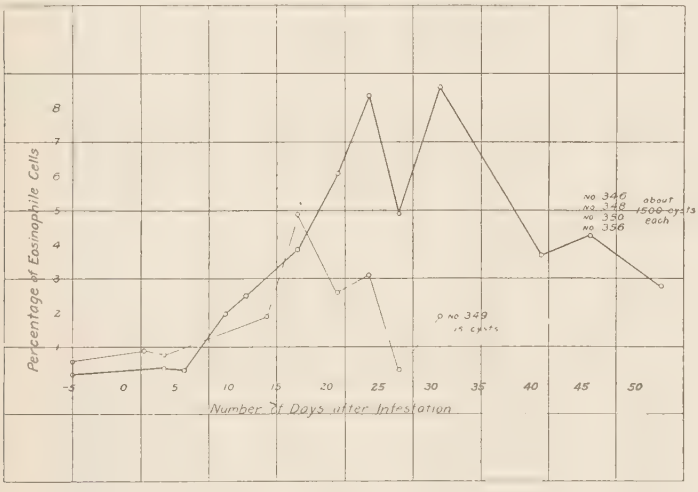


Text Figure 3.—Percentage of eosinophile cells in lightly infested rats, before and after infestation. Heavy line graph represents average for five rats. Dotted line for rat No. 299 (no cysticerci developed from 1st feeding of onchospheres) is continued as a solid line after the 37th day, when all rats were reinfested.

Experiment 3. It was evident three weeks after feeding the rats of Exp. 2 that they were lightly infested. This was probably due to the fact that it had been difficult to obtain adult *T. taeniaeformis* and that some of the proglottids used were undoubtedly immature. In order to determine what the effect of a heavier infestation would be the experiment was repeated on another group of five rats, litter mates, four females and one male; their weights ranged from 95 gms. to 102 gms. Four of these animals were fed large numbers of oncospheres, and the fifth, No. 349, female, was retained as a control; actually this control animal became accidentally infested, lightly, supposedly by means of oncospheres carried to the cage by one of the others, on its whiskers or feet; fifteen cysts were found on autopsy.

Differential counts were made before infestation, and at intervals after, for a period of 53 days (text-fig. 4). The graph for the average percentage of eosinophile cells of the four heavily infested rats (about 1500 cysts each) shows a steady rise after the sixth day, and the maintenance of a higher level, and for a longer period of time, than was found in Exp. 2. The graph for rat No. 349, which harbored only 15 cysts, begins at a higher level, and reaches its peak and drops sooner than that for the other four animals.

Judging from these two experiments the heavier infestation induces a greater reaction on the part of the rat host, but this reaction is not at all in proportion to the number of cysts present. In Exp. 2 the



Text Figure 4.—Eosinophilia in heavily infested rats. Heavy line graphs represents average for four rats; light line is for the control rat, accidentally infested with 15 cysts.

largest number of cysts was 75, while all four of the rats of Exp. 3 harbored about 1500.

Statistical analysis of the data on the percentage of eosinophilia found in infested and control rats shows that the differences are probably significant.

(1) 25 counts, uninfested rats..... mean 1.81± 0.21, δ 1.55
(The average of all data given by Donaldson for the white rat is 1.7%.)

(2) 111 counts, lightly infested rats of Exp. 2 ..... mean 2.75± 0.114, δ 1.79

(3) 67 counts, heavily infested rats of Exp. 3 ..... mean 3.33± 0.25, δ 3.03

The difference between the means (1) and (2) is 3.9 times the probable error of the difference, and that between the means (1) and (3) is 4.7 times greater than the probable error of the difference. These differences are probably significant, as the means for the infested rats are the averages of all counts, including both those before the percentage of eosinophile cells has risen and those after it had dropped.

In view of the fact that Melnikoff (1910) found different percentages of eosinophile cells in different veins of an animal after injection with extract of *Taenia solium,* two rats heavily infested with *C. fasciolaris* were examined, one 24 hours and the other six days after infestation. Differential blood counts were made from the tail, the hepatic, and the hepatic portal veins, but no significant difference in percentage of eosinophile cells was found.

Aside from the general eosinophilia which may exist, a local infiltration of eosinophile cells has been observed at the focus of infestation of many parasitic organisms, and numerous experiments have been made, especially with the injection of fluids and extracts. Bullock and Curtis (1924) mentioned finding infiltrations of small round cells and polymorphonuclear leucocytes in livers infested with *C. fasciolaris.* In the present study no local eosinophilia in the liver was found; but only sections of those containing cysts three or more millimeters in diameter were examined.

Another method of producing local eosinophilia is by the introduction of fluids or extracts of worms into the eyes of various animals. Some such fluids produce in some individuals an intense reaction, while in other cases there is no obvious effect. As a result of the experimental introduction of the fluid of *Cysticercus cellulosae* into the eyes of pigs Cauchemez (1913) ventured the opinion that the sensitiveness of some individuals is in no way related to the presence of a specific parasite.

Fluid just aspirated from living cysts of *C. fasciolaris,* in amounts varying from one to six drops, was dropped into the eyes of both infested and control rats, and examinations were made at the end of a few minutes and at half-hour intervals afterward. A few rats were thus treated soon after feeding, and again one week after infestation; in no case was there any evidence of a local reaction.

Various investigators have injected the fluids of parasitic worms, including larval cestodes, into animals, with varying results; the reasons for the lack of uniformity of effect are discussed by Simonin (1920). In the present study a dozen young rats, about forty days old, were injected intra-peritoneally or sub-cutaneously with amounts of freshly-aspirated cyst fluid varying from 0.6 cc. to 3 cc. per 100 grams of body weight; the controls were injected with equal amounts of physio-

logical salt solution. No harmful effects were observed, although the injections were repeated three times in some rats, at intervals of from nine to fifteen days.

It is well known that accidental rupture of Echinococcus cysts in man may be followed by serious, even fatal, results and that it is not necessarily infected cysts which are dangerous. The evidence indicates that this is an anaphylactic phenomenon; it is thought that the fluid may pass through the cyst wall and sensitize the host, and that anaphylaxis follows the rupture of the cyst. The fact that some cyst walls are said to be so thick as to be impermeable to the fluid may account for the cases where there are no untoward effects following rupture. Experimental anaphylaxis has also been produced by a number of investigators, using the cyst fluid of different larval cestodes; in most cases this has resulted from an injection of the fluid, some days after a previously injected, sensitizing dose. In one instance the cysts of a cestode, *Coenurus serialis,* have been punctured *in situ* in the rabbit (Henry and Ciuca, 1913) and the development of specific anti-bodies studied.

In the present investigation the puncture of numbers of cysts of *C. fasciolaris* was carried out on both lightly and heavily infested rats, numbering, in all, thirteen. These were etherized and their body cavities opened by a ventral incision; from six to forty cysts, depending upon the number present and the ease with which they were reached, were pricked with a sharp needle and the contained fluid released into the body cavity. The cysts varied in size in different rats from two to nine millimeters in diameter, and the interval between infestation and puncture varied from 17 to 130 days. No attempt was made to carry out the operations under strictly aseptic conditions; after depilation the skin was rubbed with alcohol and the incision made; this was later sutured with silk, painted with iodine, and covered with collodion. Control rats were similarly opened, the liver pricked, and the incision similarly closed up. All rats recovered very rapidly from the operation, and no differences were noted between the experimental and control animals; there was no anaphylaxis. The puncturing of 35 large cysts was repeated on two rats, 44 days after the first puncture; but again no anaphylaxis resulted.

Another phenomenon which may accompany rupture of human hydatid cyst or follow exploratory puncture is a high general eosinophilia, even up to 65%; this has been explained as due to the resorption of the cyst fluid released into the peritoneal cavity. Preliminary examinations were first made on the general stock of rats infested with *C. fasciolaris,* the differential counts being made before and after puncture of numbers of cysts; but as the results were not conclusive it was decided to repeat the experiment, using rats in which the percentage of eosin-

ophile cells had been periodically followed for more than two weeks previous to the puncturing operation. Eleven such rats were selected, and numbers of cysts opened in five of them; the remaining six were kept as controls. Differential counts were made on all rats, both operated and controls, just before the former were operated upon; and the counts were repeated 24 hours later, and in most cases two, three or four, and eight or ten days later. In no case was there a general eosinophilia produced by the released cyst fluid. In fact, there was a decided temporary drop in the percentage of eosinophile cells 24 hours after the operation, due possibly to the accumulation of these cells in the liver at or near the opened cysts, or in the peritoneum. The cysts in these rats were less than three weeks old at the time of puncture; it is possible that fluid from older cysts might have caused an eosinophilia.

The Casoni intradermal test for echinococcosis, or some modification of this test, has recently been studied by many authors, who have concluded that it is superior to the complement fixation method of diagnosis. It has also been applied in infestations of various adult helminths, but for these the reaction is not so specific; both pulverized worms and various extracts of them have been employed in the tests.

The only work on larval cestodes, other than on Echinococcus, seems to be that of Robin and Fiessinger, who made the intradermal test on guinea pigs sensitized with cyst fluid of *C. cellulosae* from man; there was no specific reaction.

Twelve rats infested in varying degrees with *C. fasciolaris* and four control animals were used in the present study. The belly wall was completely depilated the day before the experiment, and the sodium sulphide thoroughly washed off. In seven infested and three control rats 0.2 cc. of fresh cyst fluid was injected subcutaneously, forming a small bleb. In five other infested and one control rat two transverse razor cuts were made in the skin and a small portion of pulverized adult cysticerci was rubbed into the posterior cut. Examinations were made at frequent intervals during twelve hours after the experiment and again at the end of twenty-four hours; but in no case was any local skin reaction present.

The attention of early investigators was caught by the fact that there is very frequently only a single cysticercus in the liver of the wild rat or mouse. Vogel (1888) held that the presence of such an old cysticercus in the mouse conferred an immunity against a second invasion of oncospheres, and Braun thought that it appeared to be a question of the liver being able to nourish only one larva. Neither idea was accepted by later observers, not, however, on the basis of experiments. In the present study on *C. fasciolaris* the rats of Experiment 2 were success-

fully reinfested 37 days after they were first fed oncospheres, and, as noted above, both 1 mm. and 5 mm. cysts were found in the livers on autopsy.

But as these cysts were present in small numbers, another experiment, with 11 infested and 9 uninfested rats, was carried out. The interval between feedings of oncospheres was 76 days for eight of the infested rats, and 107 days for the remaining three; the cysts from the first feeding were, therefore, considerably older than those of Exp. 2 (37 days). The 8 control rats were fed the same amount of the same suspension of second-feeding oncospheres as were the experimental rats, in order to determine whether the oncospheres were viable and in this way to prevent confusion between nonviability of the oncospheres and immunity of the host to their invasion. Autopsy of the control animals showed that unfortunately the number of oncospheres given at the second feeding was too small or many of them were immature, for there were only a few cysts present in most, and none at all in two, rats. In the experimental animals from 10 to 75 very large cysts from the first feeding were present, and in only two rats were there in addition small cysts, four in one animal and twenty in another. Hence these last results are not very conclusive; but taken together with the autopsy findings of Experiment 2 it is evident that there is no absolute immunity to a second invasion of the oncospheres of *T. taeniaeformis,* at least when the cysts from the first infestation are not more than 107 days old.

A precipitin ring test was made on two occasions, using in each case one infested and one control rat's serum. The general procedure was that followed by Boyden (1926); the antigen was prepared by grinding up four grams of large, freshly-removed cysts in a glass mortar, adding 5 cc. of physiological salt solution and filtering through coarse paper and then through a fine Norton crucible. Two series of twelve tubes each were set up in each experiment, containing dilutions of the antigen from 1/20 to 1/10,240, and one tube containing physiological salt solution and another the full strength antigen. In the tubes of one series 1/12,800 antiserum from a heavily infested rat was carefully layered under the antigen, and in the control series the same dilution of serum from an uninfested rat was used; in the tube of either series which contained undiluted antigen a 1/50 dilution of serum was used. The tubes were left at room temperature and read after one-half, one, and two hours. There was no evidence of precipitin formation.

DISCUSSION

The results of the present study on the host-parasite relationship between the white rat and *Cysticercus fasciolaris* in its liver show that there is a high degree of adaptation of the host to this larval cestode.

Infestations of many degrees of intensity, even up to 6,000 cysts, have been experimentally obtained. In cases where only a small number of cysts is present there are no obvious effects on the host; and even in extremely heavy infestations the chief effect seems to be a mechanical one, the enormously distended liver pressing upon the viscera, and the function of the liver itself being affected by the cysts.

This lack of serious effect, except where abnormally large numbers of cysts are present, may be due to a number of factors. The rat is known to be a highly resistant animal, not suitable for antibody formation, and this fact may explain why anaphylaxis was never obtained, even after repeated cyst puncture. It is generally stated that the cyst fluids of larval cestodes have an excessively weak toxicity, and the effects of rupture of the cysts are believed to be due largely to anaphylaxis; but recent investigators affirm that echinococcus cyst fluid has also a variable but real toxicity, that the effects are not due to anaphylaxis alone. The fluid of the cysts of *C. fasciolaris* is plainly not strongly toxic, in fact there was no evidence of any toxicity.

Another fact which might partly explain the absence of serious ill effects is the detoxicating function of the liver in which the cysticerci are lodged. It has been found that the toxicity of certain products is diminished by passage through the liver, following injection into some part of the hepatic portal vein; if some harmful substance is present in the cyst fluid of *C. fasciolaris* and dialyzes through the cyst wall it seems possible that the liver might exert a detoxicating effect upon it.

The only very definite effect of parasitism of the white rat with *C. fasciolaris* is reflected in the percentage of eosinophile cells found in the blood. In cases of light infestation the percentage rose about two weeks after infestation and remained at a high level for about the same period; while in cases of very heavy infestation the rise began somewhat sooner and had not returned to normal after more than seven weeks. The eosinophilia of the very heavily infested rats was actually not very much higher than that found in the lightly infested ones, but both classes of rat had percentages of eosinophile cells higher than the normal. However, in no instance was there anything like the extremely high eosinophilia (even 68%) which has been found in the case of Echinococcus in man. The time of the rise in number of these cells corresponds in a general way with the peak in the cell proliferation of the host tissue around each larva.

In investigations on other parasites no correlation has ever been found between the number of parasites present and the degree of eosinophilia, possibly due to the fact that during the period of observation some parasites may have died and have been thrown out of the host, or that there may have been subsequent infestations; it is also possible

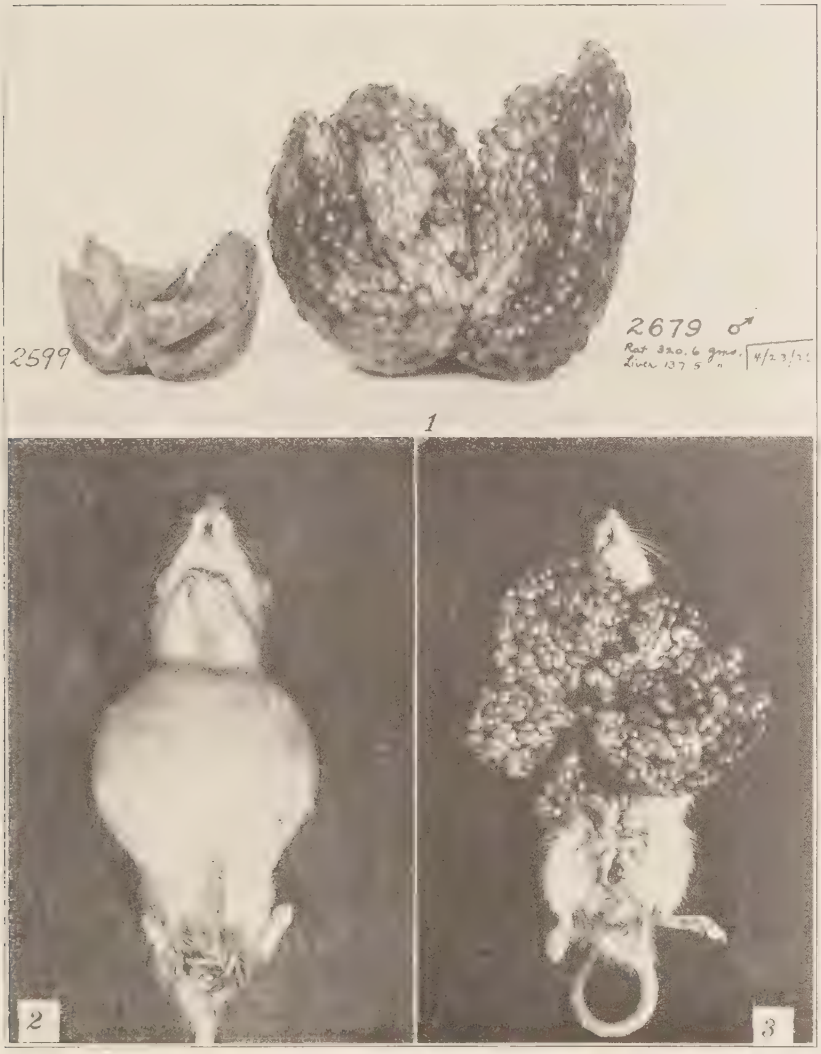


PLATE VIII

Explanation of Plate VIII

Fig. 1.—Preserved livers of two rats, brothers, of almost exactly the same body weight at time of infestation. Rat No. 2679 very heavily infested with *C. fasciolaris*, and No. 2599 kept for comparison. Both rats killed 44 days later.

No. 2599.—Total weight, 376 gms.; liver, 14.6 gms. (4% of total).

No. 2679.—Total weight, 320.6 gms.; infested liver, 137.5 gms. (43% of total).

Fig. 2.—Rat with belly wall greatly distended by heavy infestation of *C. fasciolaris* in liver; area of hair in anal region discolored by exudate from anus.

Fig. 3.—Freshly killed, very heavily infested rat, showing liver lobes turned so as to expose their dorsal surfaces; about 400 cysts visible on these surfaces alone.

that the eosinophilia found had persisted from an earlier infestation of parasites no longer present. It was expected that the present experiments with *C. fasciolaris* would show whether a correlation is present, as the material is thought to be very good for this purpose; the cysticerci cannot leave the liver and their exact number may be easily ascertained on autopsy. While the graphs (not published) showing the eosinophilia of each individual rat of Exp. 2 seem to show a rough correlation between number of cysticerci and degree of eosinophilia there is no conclusive evidence that such is the case; and the percentages of eosinophile cells in the rats of Exp. 3 are not much higher than in those of Exp. 2, although the former hosts are at least fifteen times more heavily infested.

From all evidence it would seem that *Cysticercus fasciolaris* and the white rat are very highly adapted to each other, that this cestode is a benign parasite in this host. Even when present in huge numbers the life of the host is not menaced for some months, although other investigators have found that sarcoma may eventually result from the infestation.

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A NEW STRIGEID LARVA, *NEASCUS WARDI* *

WANDA SANBORN HUNTER

Very few of the papers concerning the family Strigeidae (Holostomidae) deal with the historical background. Most of them treat the species and do not discuss the taxonomic relations. Goeze (1782) was the first to describe a species now listed as a member of this family, viz. *Hemistomum alatum,* from a fox's intestine, as a Planarian. In 1790, Abildgaard corrected Goeze's errors and described the oral and ventral suckers. Rudolphi (1802) described a second form, *Fasciola excavata.* The body form was entirely misunderstood by these three writers. Nitzsch (1816) showed that the form *F. excavata* constituted a new genus, Holostomum. He also recognized the posterior pore as the common opening of the sex organs. By 1819, six species were reported, Rudolphi discovering three new forms and Nitzsch one in this year. The adhesive organ, peculiar to the Holostomes, had been noted by Nitzsch, but his work received little attention. Not until 1828, when Blainville and Creplin agreed with him, did he receive any support. During the next few years, a small amount of unfortunately erroneous work was done: vitellaria were described as ovaries; the holdfast organ was described as a testes.

In 1845, Dujardin reviewed all of the species and established a new one. Seven species were referred to the genus Holostomum and the eighth, *H. trilobum* was erroneously referred by Rudolphi to the distomes. This description was usable except that the reproductive organs and their openings were confused. Blanchard (1847) thoroughly investigated *H. alatum* and interchanged the dorsal and ventral surfaces. In 1850, Diesing established the genus Hemistomum. He verified Dujardin's conjectures about the sex openings, but he also regarded the holdfast organ as the testes. Diesing's chief contributions were the separation of the genera, a complete bibliography of the literature and the description of four new species. Up to 1898, there followed several descriptions of single species. Diesing, 1855, gave excellent accounts of four new species. Wedl (1857) gave only an inadequate description and figure of *Holostomum trilobum* Rudolphi. Olsson (1876) wrote descriptions of *H. spathaceum* and *H. alatum.* These were first ascribed to Diplostoma since they have genital organs in the hind body. Crescentric transverse striated structures along the pharynx were designated as head glands. In the next year, von Linstow erected a new species which was found to be identical with *H. spathaceum.* Zurn (1882) in his descrip-

* Contribution from the Zoological Laboratory of the University of Illinois, under the direction of Henry B. Ward, No. 332.

tion of *H. alatum* followed Diesing and carried over his errors. Brandes (1888) published a summary of the family Holostomidae. In 1890, he produced an addition to the same work and divided the family into three subfamilies, two of which were more important, Diplostominae and Hemistominae. The Hemistominae are represented only by the genus Hemistomum Diesing. Seventeen representatives of the subfamily, Diplostominae, have been described, eleven so poorly that it is hardly possible to make a determination of them. Krause found many errors in descriptions of the anatomy. He quotes Brandes in his statement that it is remarkable that not once, among all the representatives of this family, has the outer body form been satisfactorily described.

Braun (1894) made a comprehensive presentation of the collected knowledge on the Holostomidae. He cited information concerning larval forms of two species determined experimentally by the Ehrhardt brothers. From these investigations it appears that the larval forms of the genus Hemistomum are in the genus Diplostomum von Nordmann. Railliet (1895) suggested the generic name Conchosomum for Hemistomum Diesing, 1850 (not Swainson, 1839). The meaning of the name being based upon the anterior region of the body shaped like a horn. In 1898, Stossich followed this and named two species Conchosoma.

In 1904, Johnston described two new species from Australian birds; Von Linstow (1906) described a species identical with one previously described by Brandes. In 1909, Lühe gave a short diagnosis of most of the European species. Mataré (1910) investigated for the first time an Hemistomum larva. He designated it erroneously as belonging to Tetracotyle, which, according to the Ehrhardts, is an Holostomum larva. Since Krause's excellent monograph on the Hemistominae, in 1915, Hall and Wigdor, 1918, and La Rue, 1926, have worked on this family. Railliet, 1919, named the family Strigeidae in place of Holostomidae (preoccupied) and the superfamily Strigeoidea. La Rue (1926) redescribed *Pharyngostomum cordatum* (Diesing) Ciurea 1922, and later (1926a: 11) outlined the classification of the family Strigeidae (Holostomidae) using Railliet's diagnosis of the superfamily. He also worked on the relationships of the family (1926b: 265). La Rue, Berkhout and Butler (1926) worked on the eye of fishes as an important habitat for the strigeid larvae. La Rue (1927) described a new species, *Proalaria huronensis*.

Hughes (1927) worked with the strigeid metacercariae and created a new genus, Neascus, placing particular emphasis on the reserve bladder of the excretory system. He described a new species, *Neascus ambloplitis* and suggested the possible importance of the reserve bladder in taxonomic and life-history studies. In 1928, he described *Tetracotyle pipientis* Faust (1918) and later *N. van-cleavei* (Agersborg) placing

Holostomum cuticola von Nordmann (1832), *H. brevicaudatum* von Nordmann (1832) and *H. musculicola* Waldenburg (1860) in the genus Neascus. McCoy (1928) did some work on the life history of several cercariae and found that *C. hemata* Miller (1923) develops in *Eupomotis gibbosus* into a metacercaria belonging to the larval genus Neascus.

The material used in the preparation of this paper was taken from a pond in the vicinity of Urbana, Illinois, during the year 1926-27. Eighty specimens of *Lepomis cyanellus* (Rafinesque) were examined showing an 100 per cent infection with the new strigeid metacercaria *Neascus wardi*. In order to count the cysts accurately and to show their loca-

TABLE 1.—*Infection Data*

Serial Number	Size of Fish in cm.	Cysts in Head			Mid-Region			Tail	Total Number in Host
		Dorsal	Ventral	Total	Dorsal	Ventral	Total		
1	1.9	7	11	18	11	20	31	8	57
2	2.2	7	4	11	9	10	19	1	31
3	2.3	16	6	21	11	8	19	4	44
4	2.7	20	12	32	30	27	57	20	109
5	2.7	46	68	114	55	42	97	61	272
6	2.8	35	28	63	60	43	103	45	211
7	2.8	50	60	110	83	125	208	95	413
8	2.9	25	22	47	35	27	62	7	116
9	2.9	21	30	51	30	31	61	11	123
10	2.9	35	30	65	35	23	58	18	141
11	3.0	23	30	53	25	28	53	22	128
12	3.0	30	45	75	95	30	125	10	210
13	3.1	35	40	75	55	50	105	35	215
14	3.1	60	55	115	118	89	207	90	412
15	3.2	34	37	71	40	25	65	13	149
16	3.3	30	25	55	40	45	85	11	151
17	3.5	25	20	45	37	40	77	25	147
18	3.5	30	35	65	70	45	115	25	205
19	3.5	45	36	81	85	60	145	40	266
20	3.6	38	30	68	22	40	62	16	146
21	3.6	30	32	62	40	32	72	12	146
22	3.7	40	42	82	80	30	110	25	217
23	3.7	60	54	114	75	40	115	30	259
24	3.8	30	21	51	52	48	100	36	187
25	3.8	40	67	107	55	49	104	28	239
26	3.8	60	49	109	100	65	165	43	317
27	4.0	16	36	52	56	40	96	20	168
28	4.4	30	40	70	45	50	95	25	190
29	4.5	45	60	105	60	75	135	20	260
30	5.1	45	80	125	110	59	169	30	324
31	5.6	75	80	155	105	150	255	85	495

tion, 31 of the fish were cut into three pieces, the first cut being immediately posterior to the operculum, and the second being immediately posterior to the junction of the anal fin. The head and mid regions were then cut along the longitudinal axis so as to separate each into a dorsal and a ventral half; the tail region not being divided. It is interesting to note the numbers of cysts found in each of the resulting five regions (see Table 1).

There has been a question in the writer's mind concerning the correlation between the number of cysts found in the host and the size of the fish examined. McCoy (1928) found that a very heavy infestation of the fish with metacercariae does not build up a resistance to a second infestation. There seems to be a definite correlation, however, between

the number of cysts in a host and the size of the fish examined. A fish 1.9 cm. long had only 57 cysts and one 5.6 cm. in length had 495 cysts. The correlation is no doubt due to the difference in the ages of the fish and the greater number of infestations which the older fish encounters.

There are two cysts surrounding each larva. The outer are usually elliptical in shape, measuring from 510 to 850μ in length when not subjected to pressure. This outer cyst is characterized by a heavy black pigment. The ovoid inner cysts in which the larvae are folded is tough, transparent and membraneous. These measure from 300 to 340μ and are usually surrounded by a gelatinous mass. The inner cyst is very flexible, shaping itself to the active larva within. The description of the parasite is based almost entirely upon preserved material, both in toto mounts and sectioned larvae. The excretory system and general characteristics were obtained from live material. The larvae studied were dissected from the cysts with needles; about 10 per cent of the larvae

TABLE 2.—*Measurements of Preserved Specimens in Microns*

	Number Measured	Minimum	Maximum
Length of forebody	18	196	473
Length of hindbody	17	115	419
Total length	19	378	817
Width of forebody	20	95	351
Width of hindbody	17	41	236
Diameter of oral sucker	20	40	79
Depth of oral sucker	13	37	70
Diameter of oral cavity	12	14	56
Diameter of acetabulum	16	22	43
Position of acetabulum (from anterior end of forebody)	17	115	290
Diameter of adhesive organ	17	40	79

emerged from the cysts when placed in a normal saline solution at room temperature. The trypsin-soda method described by Hughes (1927) was tried without success. The dissection was greatly facilitated when the cysts were allowed to stand in the saline solution from 4 to 8 hours.

The body of the larva is clearly constricted into two parts, a flat, leaf-shaped forebody with the lateral and posterior edges cupped ventrally and a hindbody which is conical in shape. The total length of the animal varies from 378 to 817μ, the forebody measuring from 196 to 473μ and the hindbody from 115 to 419μ (see Table 2). The width of the forebody in the region of the adhesive organ varies from 95 to 351μ, the width of the hindbody ranging from 41 to 236μ in the widest part. These measurements were taken from toto mounts and the great amount of variation is caused by the various states of contraction of the larvae.

The strong muscular oral sucker, ventro-terminal in position, measures rom 40 to 79μ in diameter and from 37 to 70μ in depth; the shape varies from round to oval. The chief muscles of this organ are radial, the long slender fibers flaring at both ends. Among them are scattered large myoblasts which are irregular in outline. One of these

myoblasts was clearly seen in cross section and it measured from 3 to 4μ across. A narrow band of circular muscles surrounds the radial fibers which measures no more than 1μ at its widest part (Fig. 1). The acetabulum is partially embedded in the ventral surface of the anterior region of the posterior half of the forebody, the actual measurements being 115 to 290μ from the anterior extremity of the body (Fig. 2). The diameter varies from 22 to 43μ and the depth is from 24 to 30μ. The chief muscles as in the oral sucker are the radial. These, however, are not as heavy nor as close together as those of the larger oral sucker. Large dark nuclei are found scattered among the muscle fibers at the base of the organ (Fig. 3).

The large adhesive organ is posterior to the acetabulum, protruding from the anterior region of the posterior fourth of the forebody. It has a variety of shapes inasmuch as it is very contractile and is almost always in motion. While studying the living specimens, it has been observed to flatten out, to become pointed, and to move in all directions. When at rest, however, it resembles an inverted cone measuring from 40 to 79μ in diameter. The opening of the cleft is located in the center of the ventral surface; it measures from 24 to 36μ in length and from 7 to 8μ in width. The cleft is branched near the base of the organ and the two primary branches divide into several smaller tubes which lead dorsally (Fig. 4). The glandular walls of the cleft stain deeply and are 2 to 3μ in thickness, the cavity itself being 3 to 4μ wide. Many deep staining nuclei are found throughout the tissues of the vesicle, particularly in the dorsal and ventral regions; these are undoubtedly glandular in function. The adhesive organ is from 22 to 53μ in depth. Specialized muscle fibers are seen in the outer wall and through the main part of the organ leading from the layer of circular muscles and the flat ventral surface to the base. Circular muscles surround the surficial opening and the longitudinal muscles.

There are three bodies located in the posterior half of the forebody which are believed to be the rudiments of the reproductive system. The largest of these bodies is the ovary which is immediately dorsal to the acetabulum. It is disc-shaped and appears slightly lobate in sagittal section. The length of this body is from 32 to 46μ and it is from 8 to 18μ in thickness. The other two bodies, or testes, are smaller and are found, widely separated yet paralleling each other posterior to the organ of adhesion. In sagittal section these measure from 14 to 28μ in length and from 4 to 6μ in width. In toto mounts these three bodies appear as aggregations of large nuclei but in sections as clearly defined dark staining cell masses each having a thin membrane surrounding it (Fig. 5).

In the posterior fourth of the hindbody between the digestive crura is found a mass of large heavily staining nuclei (Figs. 2, 6). This mass,

in toto mounts, appears spherical, sometimes elongated, never regular, and measures from 30 to 90μ in width by 46 to 64μ in length. It is found in the region one would expect to find a bursa copulatrix and is no doubt the primordium of that organ. A smooth non-striated and non-spinous cuticula, measuring about 1μ in thickness covers the entire body. Immediately under the cuticula is found an exceedingly thin layer, or basement membrane, which stains a deep pink like the parenchyma (Fig. 7).

Next to the basement membrane is found a thin layer of circular muscles, the maximum width of which is 1μ. Within this layer, and close to it, are the longitudinal muscles, which extend the length of the entire body. This layer measures from 1 to 2μ. Inside the longitudinal muscles, and with their flaring ends adjoining it are found the radial, or dorso-ventral muscles. These fibers are long and extend into the parenchyma. The nuclei of these muscles are clearly seen in sections. The parenchyma of this larva is an evenly distributed, fine substance completely filling the body and forming a matrix in which the internal organs are located. It takes a deep eosin counterstain. Many large cells probably glandular in function, are scattered throughout this matrix; the cell walls take a deep stain as well as the nuclei and cellular inclusions. These cells tend to aggregate near the periphery of the parasite although they are found scattered through the deep parenchyma in all parts.

The simple digestive system consists of an oral cavity followed by a muscular pharynx which in turn leads into an esophagus. The esophagus bifurcates into two lateral ceca. The bifurcations are not exceedingly far apart but seem to spread out gradually. They are blind tubes extending nearly to the posterior end of the hindbody (Fig. 2). This system is partially lined with a thin cuticula which seems to be a continuation of that of the outer body covering. It differs in thickness from that of the outer covering, and reacts to the stain in the same manner. This cuticula does not extend beyond the extreme anterior end of the intestinal crura. The tubes of this system are sometimes filled with a grayish, grandular appearing substance or a dark blue staining grandular substance (Fig. 6).

The oral cavity is surrounded by the large muscular oral sucker described in the preceding part. It measures anywhere from 14 to 56μ in diameter in the toto mounts, the width depending upon the state of contraction of the sucker. A cuticula measuring at most 1μ in thickness lines the cavity which ends at the posterior margin of the sucker. The oral cavity leads posteriorly and slightly dorsally into a muscular pharynx, the diameter of which varies from 16 to 20μ. The walls of this pharynx are about 8μ in thickness, and are lined with a thin cuticula.

The length is from 20 to 25μ in the sections measured. The muscles of the pharynx are similar to those of the oral sucker; the radial muscles, however, are smaller or slightly finer fibers than those of the sucker. The circular muscle layer measures less than 1μ in thickness.

The pharynx leads into a median esophagus 9 to 12μ in diameter, the walls of this part being 3μ in thickness and non-muscular. It is also lined with an exceedingly thin cuticula. The esophagus extends beyond the pharynx for 10 to 15μ before it bifurcates. The bifurcations or digestive ceca vary from 4 to 8μ in diameter. The walls are less than 1μ in thickness. These two straight tubes lead posteriorly, one on either side of the acetabulum, and end blindly in the posterior region of the hindbody. The cuticula is not found lining these bifurcations except at the extreme anterior end, immediately posterior to the branching. The process of absorption takes place in the ceca, and if a cuticular lining were present in them, the process would be hindered even though the lining were very thin.

In studying the excretory system, no flame cell pattern was traceable and the reserve bladder is the only part of the system which has been worked out. The medium dorsal vessel (Fig. 8 *m*) extending from a point immediately posterior to the oral sucker, divides in the regions of the pharynx, acetabulum and adhesive organs to form a pharyngeal ring (*pr*), an acetabular ring (*ar*) and a ring around the adhesive organ (*ad r*). Within the two posterior rings are networks of smaller vessels which are connected with the larger vessels (Figs. 4, 8). These two posterior rings are also broadly connected with each other. There are usually four pairs of transverse commissural vessels (*tc*) connecting the median dorsal vessel with the primary lateral vessels in the anterior region of the forebody. One pair of lateral vessels (*vl*) and a pair of extra-lateral vessels (*el*) is found running posteriorly from the region of the second pair of transverse commissural vessels. A pair of intralateral vessels (*il*) leads posteriorly from the fourth pair of transverse commissural vessels. From these last transverse commissural vessels, there is also a pair (*ta*) lying near the median dorsal vessel, which connect with the acetabular ring. The marginal vessel (*mv*) is a small closed vessel running near the entire margin of the forebody. It is connected to the larger collecting vessels by numerous anastomoses. The main vessels in the forebody are all connected by many small anastomoses, all of which cannot be seen without repeated observations and which show individual differences in various specimens. Near the posterior margin of the adhesive organ, the lateral vessels form one lateral collecting vessel (*lc*) on either side. These join just anterior to the constriction of the fore and hind bodies and the resulting median ventral vessel (*vv*) continues posteriorly for about one-third the length of the hindbody.

Here two ventro-lateral vessels (*vl*) are formed which continue to the posterior region of the hindbody. They enlarge and unite to form a small contractile urinary bladder (*u*) which opens to the exterior by means of a short terminal excretory duct (*ed*) and a terminal excretory pore. The median dorsal vessel continues from the ring around the adhesive organ to the point where the median ventral vessel divides. Two dorso-lateral vessels (*dl*) are formed in this region and continue posteriorly until they lead into the posterior dorsal surface of the urinary bladder. There are from six to ten semicircular commissural vessels (*sc*) connecting the median vessels in the hindbody. There are from seven to fourteen such vessels connecting the dorso and ventro-lateral vessels. These small connecting vessels lie close to the surface and are external in relation to the larger vessels. The lateral vessels were clearly seen to branch medianly, but the branches were not traceable. There is a coarse network of fine vessels immediately under the surface of the hindbody which is probably connected to the semi-circular connecting vessels and the main vessels. The reserve bladder is filled with a fluid in which many small calcareous granules are found. The granules are moved about freely in the fluid and were seen leaving the body through the excretory pore. The calcareous nature of the granules was determined by treatment with dilute hydrochloric acid.

The nervous system consists of a supra-pharyngeal commissure and six main branches. From the ventral surface, it has an H-shaped appearance, with the bar of the H being at the anterior end, in the region of the pharynx and esophagus. The branches are paired, being divided into two lateral groups with a dorsal, ventral, and lateral branch on either side. The ventral branches are the largest and these end anteriorly at the commissure. The dorsal and lateral branches go forward towards the oral sucker and then curve backwards extending to the posterior sixth of the forebody. In the anterior end, narrow cross connections are found between the branches which give the system a ladder-like appearance (Fig. 2). The supra-pharyngeal commissure measures from 36 to 42μ in width and from 4 to 7μ in thickness. The ventral branch is from 4 to 6μ wide and extends to the posterior end of the body. The two central branches are 36 to 58μ apart in the anterior region, and from 80 to 90μ apart behind the acetabulum. The ventral and dorsal branches are about 16μ apart and there is from 16 to 18μ between the dorsal and lateral branches. The narrow connections joining the main branches of this system are found to be evenly spaced, at about 12μ intervals along the ventral branches.

The reasons for placing the described larva in this genus are briefly summed up as follows: The specimens are encysted strigeid metacercariae with the fore and hind bodies clearly constricted. No lateral

sucking cups are present. The forebody is leaf-shaped. There is a well developed holdfast organ. The reserve bladder of the excretory system forms a prominent network throughout the body, and numerous calcareous granules are free in the fluid within this reserve bladder. It is classed as a new species because of very marked differences from the five previously described species of Neascus. These differences are brought out by comparisons with each of the species described (Hughes, 1928). *Neascus wardi* differs from *N. cuticola* (von Nordmann) in that the inner cyst is ovoid, the acetabulum is smaller than the oral sucker, the intestinal ceca in the hindbody are relatively small, and the reserve bladder is peculiar to this species. It differs from *N. ambloplitis* Hughes in that the ventral surface is non-spinous, the oral sucker is not ridged, the acetabulum is small and apparently almost vestigeal. No bursa copulatrix is developed. The differences in the reserve bladder are very noticeable, there being a pharyngeal ring in *N. wardi,* direct connections between the fourth transverse-commissural vessel and the acetabular ring and other minor differences which are shown in the diagram. The reproductive organs are in the forebody.

N. wardi may be distinguished from *N. van-cleavei* (Agersborg) by the cysts which are black-pigmented, thick walled, in the skin, flesh and fins; the parasite is folded within the cyst; the constriction between the fore and hind bodies is continuous around the entire body; the forebody is not coarsely striated due to large inner longitudinal muscle fibers; the acetabulum is smaller than the oral sucker; no fundaments of vitellaria are evident; the reproductive organs are in the forebody; and lastly, the pattern of the reserve bladder is markedly different. In comparing this species with *N. musculicola* (Waldenburg) the characteristics which stand out as peculiar are the pigmented cysts, the body folded within the cyst and the size of the hindbody. The hindbody of *N. wardi* is relatively smaller than the forebody while that of *N. musculicola* is relatively larger than the forebody.

N. wardi is an encysted form, the forebody is leaf-shaped and not narrowed posteriorly in comparison with the non-encysted larva and the heart-shaped and posteriorly narrowed forebody of *N. brevicaudatus* (von Nordmann). *N. wardi* may be considered as a new species primarily because of the location of the reproductive organs. These three cell masses, surrounded by thin membranes, are rudimentary; there are no connections between them and no tubes found developing from them. Except for their location they closely resemble the reproductive organs of *N. van-cleavei.* They cannot be adhesive glands because there are no connections with the adhesive organ, they are not in positions logical for such a function, and a glandular mass of cells appears at the base of the adhesive organ to supplement the glandular walls of the cleft.

Morphologically and histologically they are reproductive organs. Their number and relative sizes would also place them as such. Furthermore, no traces of any organs besides that described as the primordium of the bursa copulatrix have been found in the hindbody.

I wish to express my appreciation to Dr. Henry B. Ward of the Zoology Department of the University of Illinois under whose direction this work was done and Professor A. W. Bray and Dr. George W. Hunter, III, of Rensselaer Polytechnic Institute for the use of their laboratories and equipment during the completion of the paper.

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Abbreviations

a—acetabulum
ad—adhesive organ
an—network in acetabular ring
ar—acetabular ring
adn—network in adhesive organ ring
adr—ring around adhesive organ
b—primordium of bursa copulatorix
c—cleft of adhesive organ
d—digestive cecum
dl—dorso-lateral vessel
e—excretory duct
ed—terminal excretory duct
el—extra-lateral vessel
il—intra-lateral vessel
lc—lateral collecting vessel
lv—lateral vessel
m—median dorsal vessel
mv—marginal vessel
n—nervous system
o—ovary
p—pharynx
pr—pharyngeal ring
s—oral sucker
sc—semicircular commissural vessel
t—testis
ta—vessel connecting transverse commissural vessel with acetabular ring
tc—transverse commissural vessel
u—urinary bladder
vl—ventral lateral vessel
vv—median ventral vessel

The lines in all of the figures have a value of 0.01 mm. except in figure 5 which has a value of 0.1 mm.

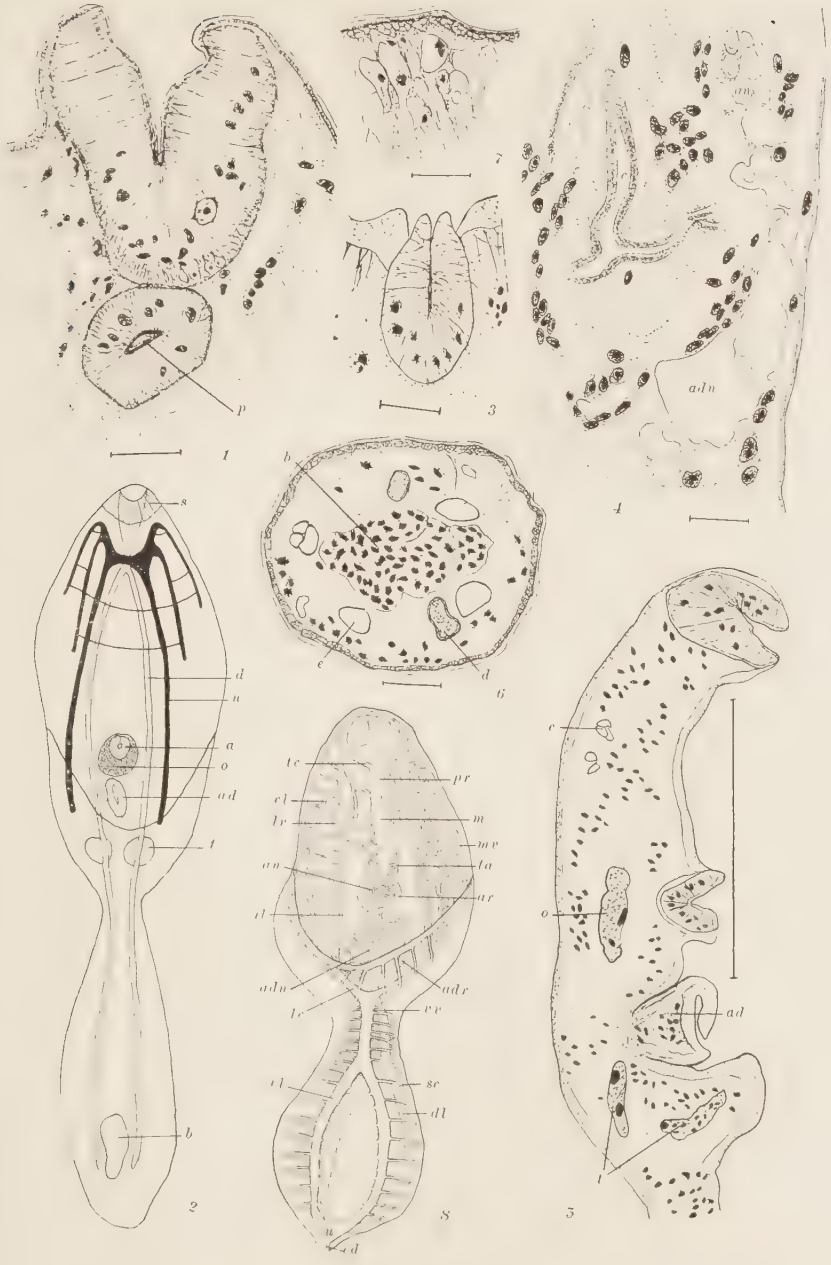

PLATE IX

Explanation of Plate IX

Fig. 1.—Cross section through oral sucker and pharynx.

Fig. 2.—Diagrammatic reconstruction of toto.

Fig. 3.—Cross section through acetabulum.

Fig. 4.—Sagittal section through cleft of adhesive organ and ring around adhesive organ.

Fig. 5.—Sagittal section through forebody.

Fig. 6.—Cross section through primordium of bursa copulatorix.

Fig. 7.—Cross section through integument.

Fig. 8.—Outline sketch of reserve bladder drawn from living specimens.

ON THE GEOGRAPHICAL DISTRIBUTION AND AFFINITY OF THE APPENDICULATE TREMATODES PARASITIZING MARINE PLANKTON COPEPODS

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If (as will be shown later on) Claus (1863) is not to be regarded as the discoverer of the young appendiculate trematodes living in marine plankton copepods as intermediate hosts, a copepod infested by such parasites seems to have been found for the first time in the Bay of Kiel by Möbius ("der nichts darüber publiziert hat" Willemoes-Suhm, 1871) and is to be identified as *"Distoma ocreatum* Rud. der Heringe." Willemoes-Suhm then observed 1871, "ein freilebendes geschlechtsloses Distom" in the Baltic Sea as well as in the Sound from the middle of June very frequently and found that it fastens "an Wurmlarven und Copepoden." Again in the Bay of Kiel Giesbrecht (1882) mentions the frequency of the parasite in higher stages of development in mature individuals of the species *Pseudocalanus elongatus* Boeck (syn. *Lucullus acuspes* Giesbr.) ; but also it has several times been found free-living. Only rarely has *Centropages hamatus* (Lillj.) been infected by it, very likely because then (February 1880) *Pseudocalanus elongatus* was the most frequent free living copepod. Later on these trematodes from Kiel have been more closely described and figured by Monticelli (1891) as young forms of *Apoblema (Distoma) appendiculatum* (Rud.). By this name they are also mentioned by Canu (1892) as parasites of *Paracalanus parvus* (Cls.) and *Acartia clausi* Giesbr. from Wimereux, as well as by H. S. Pratt (1899) as "Appendiculate Distomes" from Long Island Sound, that is from the eastern coast of North America. They have been found there in summer from the middle of July, first scarce and free living, then more frequently till August in copepods not more closely determined. Richard (1899) notes as parasites of *Centropages hamatus* (Lillj.) (syn. *Ichthyophorba angustata* Cls.) a *Distomum ichthyophorbae* Grebint, of which I have not been able to find any details up to the present. Apstein (1911) mentions several localities from the North Sea: Kattegat, the Great Belt, Belt Sea and Baltic Sea, where he could demonstrate "Distomen" (with reference to the often mentioned *Distomum appendiculatum* Rud.) in *Calanus finmarchicus* (Gm.), *Pseudocalanus elongatus* Boeck, *Paracalanus parvus* (Cls.), *Temora longicornis* (Müll.), *Centropages hamatus* Lillj. and Acartia sp.

From the western coast of Scotland (Firth of Clyde) S. M. Marshall (1925) states, that she seldom found *Hemiurus appendiculatus* in *Acartia clausi* Giesbr. and in *Calanus* sp. At the same time Rose (1924) reports in his work on the pelagic copepods of the French coasts that he very often found "Metacercaria of Distomes," "parfois jusque dans l'antenne préhensile du mâle; mais a Roscoff seulement." The trematodes living on or in Acartia he calls (1925) Apoblema and found up to three in one and the same individual. It is striking that Rose, who evidently paid great attention to parasites in his plankton researches, has not been able to discover the worm either on the French Mediterranean or on that of Algiers, but only in the Channel, both times evidently near Roscoff (which he finds necessary to remark especially for Centropages).

Thus there never seems to have been found as yet in the Mediterranean a copepod infected by the trematode in question, which is also in accordance with the information of Messrs. Dr. R. Dohrn and Tregouboff in Naples and Villefranche. At the Naples Station the parasite has not been especially looked for; Tregouboff, however, writes distinctly: "malgré que je fasse toujours attention aux parasites des animaux pélagiques, je n'ai jamais rencontré dans notre region le Distome en question."

Canu has good reason to suppose (1892) that the "Monostomum" out of the body cavity of *Paracalanus parvus,* represented by Claus (1863, Pl. 27, Fig. 2) is to be identified with our appendiculate Distomum. This would then really be the first discovery, unfortunately without stating the locality. Claus, however, states in the preface, that his material is partly from the Mediterranean (Messina, Naples, Nice), partly from the North Sea (Helgoland), hence only Helgoland can come into question for this trematode.

Thus free living, pelagic copepods infected with young distomes seem to have been found only in the North European seas (Northern Channel of the Irish Sea, Channel, North Sea to the Western Baltic) and besides on the eastern coast of North America. All these localities, however, are within the zone of distribution of the herring and the sprat (A. Meek, 1916).

According to Looss (1907) the following species of the genus Hemiurus are known in the adult state: *H. appendiculatus* Rud., "exclusively in *Alosa finta*" (syn. *Clupea finta* Cuv.). According to Meek (1906: 105) the Twaite Shad "is more southern in distribution than the Herring," and according to D'Ancona a difference is to be distinguished between a northern and a Mediterranean biological local race (1927). Their food seems to consist especially in the young state, of small (pelagic) animals; in the estuaries of the northern seas especially the copepod *Eurytemora affinis* (Poppe) is said to be of great importance

on account of its great abundance (Kyle und Ehrenbaum, 1927). Th. Scott (Sep. without date) does not mention it. In the Mediterranean the adult Fints seem to feed more on Benthos-animals (Gammarini), as D'Ancona (1927) says.

H. lühei Odhner, 1905, lives in *Clupea harengus* and *sprattus*. The food of the herring, however, consists, as Jespersen (1928) shows in the Danish herring, of more than 47% of free living copepods, among which, it is interesting to state, *Pseudocalanus elongatus* and *Paracalanus parvus* form over 80% of the total copepods found in the herring of Samsö. *Pseudocalanus elongatus* is a spring form in the Samsöplankton (April-June), *Paracalanus parvus* an autumn form (September-November). In both species, however, the appendiculate distomes in question have been found most frequently (*Pseudoc. elong.* by Giesbrecht and Apstein, *Parac. parv.* by Canu, Claus and Apstein). Then there follow: *Centropages hamatus,* only 3% of all copepods (especially in spring), and also especially in spring; *Temora longicornis,* more than 14% of all copepod food of the Danish herring. Distomes have been found in *Centr. ham.* by Giesbrecht and Apstein, and in *Temora longic.* by Apstein.

This most striking parallelism between frequency as herring food and frequency of infection with distomes leads to the supposition that the appendiculate distomes in copepods are to be attributed to the species *Hemiurus lühei* and not, as has been believed heretofore, to *Hemiurus appendiculatus.*

Perhaps one might also find relations as to the occurrence at certain seasons, which aspect up to the present time has not had attention. *H. rugosus* Looss 1907 lives according to Looss in *"C. pilchardus* and *Clupea sardina"* (sic!) and is therefore limited to the region of distribution of the sardine. A specimen in my collection, 0.81 mm. long, from a pilchard, from Rovigno in the year 1907, is according to the determination of Miss Lebour also of this species.*

Of less importance here are: *H. communis* Odhner 1905 and *H. levinseni* Odhner 1905, which both live in *Gadus morrhua* and other northern fish. The principal food of the cod up to a length of about 15 cm. consists according to Schnakenbeck (1926) especially of amphipods. "Erst nach einer Länge um 60 cm. ab überwiegen die Fische in der Nahrung." With *H. communis,* however, Odhner (1905) wants to identify the North American Hemiurus described by Pratt (1889). "Nur ihre ungeteilte Samenblase spricht gegen eine solche Identifizierung," and according to Miss Lebour's amiable communication the

* Miss Lebour (Plymouth) being a specialist, had the kindness to revise the manuscript and to correct the English translation, for which I thank her most heartily in this place.

distomes found on Acartia at Plymouth also belong to *H. communis* Odhner. This species is the most common in Northumberland as well as in Plymouth "although *H. lühei* has also occurred in Plymouth fishes, especially in the Pilchard."

Young appendiculate distomes have also been found in Beroë (Will, 1844), larvae of annelids and in chaetognaths, especially in Sagitta. Recently an adult Distomum has been described from the gut of *Sagitta elegans* by Linton (1927). Although Sagitta is very common in Danish waters at certain seasons (especially August-October) it is astonishing how seldom they are eaten by herring (3.1% to 74.4% copepods); only in the North Sea the chaetognaths seem to be of greater importance as herring food during winter (Jespersen, 1928). As to their food

Distribution of Copepods infected by Hemiurus.

Apstein says (Bull. Trim. p. 171): "Die Chaetognathen sind Räuber, die mit ihren starken Greifhaken Copepoden festhalten und verschlingen. Mit den Copepoden nehmen sie auch deren zahlreiche Parasiten auf, die bei ihnen aber naturgemäss sich im Darm befinden. Ob die Parasiten in den Chaetognathen einen Zwischenwirt finden, oder in ihnen zugrunde gehen, kann ich nicht angeben. Ich fand die Parasiten lebend in den Chaetognathen, sie könnten aber auch erst seit kurzem in dieselben hineingekommen sein." By my observations in the Adriatic the relative frequency of infected Sagittas tells against the supposition that the trematodes die in them, for the copepods are swallowed undivided and the trematodes can easily free themselves in the gut of the chaetognath. Pratt (1898) had already noticed their "great vitality." Now Apstein says as to the distomes found in Sagitta of the North Sea, Kattegat and

the Belt Sea: "Ich kann nicht entscheiden, ob es sich um die Art aus den Copepoden handelt oder ob es eigene Parasiten der Sagitten waren." Busch (1851) quotes three distomes living in "Sagitta." According to Lebour (1916-1918), however, it is *Spadella cephaloptera;* two of the three distomes found therein are very likely really larvae of Hemiurus. In Sagitta of Plymouth Sound Lebour found: *Pharyngora bacillaris* (Molin) "without a tail" and *Derogenes varicus* (O. F. Müll.). The former species has been discovered by Molin (1859) in *Centrolophus pompilius* in Padua, the latter occurs according to Odhner (1905) in the adult state in Sturgeons, Pleuronectids, Labrids, Cottids, Gadoids, that is, especially in bottom fishes "was vermutlich mit der Lebensweise des Zwischenwirtes in Zusammenhang steht," which is said to be a polychaet *Harmothoe imbricata.* Derogenes is systemtaically near the Hemiurids s. str.

From the Mediterranean I only know of Sagittas from the Adriatic infected by appendiculate distomes, namely, from the Gulf of Trieste (from two stations) and the Drin-Gulf (from one station). In the Gulf of Trieste I could observe them from April to November. I only met them once free in the plankton in April, 1903, and twice in August, 1902 (Steuer, 1910).

One Hemiurus-larva, 0.33 mm. long, found in an Adriatic Sagitta on Sept. 1st, 1927, belongs, according to Miss Lebour's kind determination very likely to *H. rugosus* Looss. I never found during the expeditions on H. M. S. Najade infected Sagittas on the high seas. It is striking in this connection that Chaetognaths perform an important rôle in nourishing the Adriatic sardine (Steuer, 1908). According to these facts Sagitta may be regarded as intermediate host of the *Hemiurus rugosus* living in the pilchard.

It is my task to point out that in deciding difficult parasitological questions the geographical distribution of the parasites, their hosts and intermediate hosts play a rôle not to be neglected, which has not always had the requisite attention. According to our present knowledge *the trematodes parasitising copepods are not the young of Hemiurus appendiculatus but they belong, as far as they occur in the northern seas more likely to H. lühei and communis. In the Mediterranean the worms in question have as yet never been found in copepods but principally in chaetognaths (Sagitta) or free in the sea and very likely belong to the species H. rugosus, which in the adult state parasitises the intestine of the pilchard.*

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SEASONAL FLUCTUATION IN THE INFESTATION OF *PLANORBIS TRIVOLVIS* WITH LARVAL TREMATODES *

OLIVER R. MCCOY

Practically no attention has been given to the seasonal fluctuation in the degree of infestation of snails with larval trematodes. It has often been suggested that there is seasonal variation in the appearance of cercariae in their molluscan hosts but only a few extensive surveys have been made to determine the amount and nature of the variation.

The first extended observation of seasonal infestation with larval trematodes was made in the study of schistosomiasis in Egypt by Manson-Bahr and Fairley (1920) who kept a record of the cercariae found in the snails which they dissected over a period of one year. They found the cercariae of the human blood flukes, *Schistosoma haematobium* and *S. mansoni,* present in the snails at all seasons of the year. The highest percentage of infested snails occurred during the month of December. They note also that the infestations found in the autumn months were all mature, whereas in the spring months immature cercariae were the rule rather than the exception. Their observations on the other larval trematodes found in the snails indicate the same general trend, for the snails were infested throughout the year, with the highest percentage occurring in December. Their study was made in a part of the world in which drought has a great influence on the snail population and where there is not a marked fluctuation in temperature from season to season.

Soparkar (1921) published a two year record of the prevalence of the carcaria of *Schistosoma spindale* in the Indian fresh-water snail, *Planorbis exustis,* and found the percentage of infested snails highest in the autumn months and lowest during late winter and early spring. His observations were based on 25,000 individuals, but he recorded only the cercariae which emerged from the snails and did not dissect the snails to determine the total infestation.

Sewell (1922) reported a curve of the infestation with larval trematodes for the Indian fresh-water snail, *Melanoides tuberculatus,* in which the high points were in December and July. Sewell's curve, however, was based on only 139 snails. The most complete survey of the seasonal

* This investigation was conducted in the Zoological Laboratory of Washington University, St. Louis, from October, 1925, to October, 1927, under the direction of Dr. H. M. Miller, Jr., whom the writer takes great pleasure in thanking for his friendly interest in the work and his many valuable criticisms and suggestions.

fluctuation in the appearance of trematode cercariae was made by Miller and Northup (1926) over a 12 month period on the marine snail, *Nassa obsoleta,* from Woods Hole, Massachusetts. They examined over 8,000 specimens and found a definite semi-annual rise and fall in the infestation, the high points occurring in December and July. In their study the percentage of parasitized snails was quite low, ranging from 2.3 to 8.5%. Various isolated observations have been recorded on the seasonal occurrence of larval trematodes but records of no other extended surveys have been found.

A knowledge of the seasonal appearance of larval trematodes would not only suggest their probable life cycles but might also throw light on some general points upon which very little is known. Such problems, for example, as the length of time that the larvae parasitize the snails, or their possibility of surviving the winter in the molluscan host have never been definitely worked out. The purpose of this survey was to observe over a two-year period the seasonal infestation with larval trematodes of a fresh-water snail, *Planorbis trivolvis* Say, from a limited locality.

Snails were collected from Ramona Park Lake near St. Louis, Missouri, at intervals of from three to four weeks. The lake, which is about 150 yards long and 50 yards wide, is artificial but has been in existence for over 30 years. *Planorbis trivolvis* and *Physa integra* Haldeman are the only species of snail present. Four species of fish, and muskrats, turtles, snakes, frogs, and numerous migratory birds are possible vertebrate hosts known to live in or frequent the lake. The area is closely circumscribed, for no streams flow into the lake and no other bodies of water are near it.

Twenty-nine collections of *P. trivolvis* have been made over the two year period and a total of over 6,500 snails examined. *P. trivolvis* was abundant in the spring, summer, and fall and there was no difficulty in collecting several hundred specimens on each trip. The snails were always taken from only one side of the lake and as far as possible full-grown individuals were selected. In the winter, collections were made by breaking through the ice and it was sometimes difficult to obtain a large number. The snails were brought into the laboratory and isolated in glass vials. If no cercariae emerged in 48 hours, the snails were killed and dissected. Any infestation which did not give emerged cercariae in 48 hours was considered to be immature regardless of whether or not on dissection of the snail the cercariae appeared to be fully developed.

In the course of the study, six different species of cercariae were found in *P. trivolvis* but of these, only two were sufficiently abundant to furnish adequate data for observations on the seasonal infestation. The complete data of the occurrence of these two species, *Cercaria hamata*

Miller, 1923, and the cercaria of *Plagiorchis ameiurensis* McCoy, 1928, are given in the table.

The percentage of the snails in each collection which were infested with these two species are plotted in Figure 1, curves II and V. The percentage of the snails examined which harbored mature infestations, i. e., cercariae emerged in 48 hours, is also given in Figure 1, curves III and VI. The former curves show the prevalence of the cercariae at any certain season of the year, while the latter show the percentage of snails in which the cercariae were fully developed. A more definite indication of the seasonal cycle, however, is given by the consideration of the percentage of the infested snails in each collection in which the cercariae were mature. For instance, if in one collection, all of the infestations are immature, the indication is that the snails have recently been infected

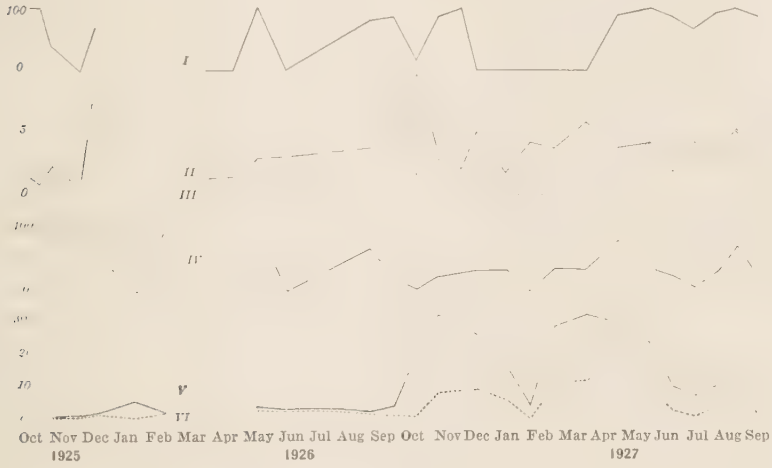

Figure 1.—Graphs showing the infestation with larval trematodes of *Planorbis trivolvis* from October, 1925, to October, 1927.

I. *Cercaria hamata,* percentage of the infested snails which gave emerged cercariae.

II. Percentage of snails infested with *C. hamata.*

III. Percentage of the snails harboring mature infestations of *C. hamata.*

IV. The cercaria of *Plagiorchis ameiurensis,* percentage of the infested snails which gave emerged cercariae.

V. Percentage of snails infested with the cercaria of *P. ameiurensis.*

VI. Percentage of the snails harboring mature infestations of the cercaria of *P. ameiurensis.*

and that sufficient time has not elapsed for cercariae to mature. On the other hand if all are mature, it is probable that no new infection has taken place. To illustrate this relationship curves have been plotted of the percentage of the infestations in each collection which were mature (Figure 1, curves I and IV).

The first of the two cercariae to be considered is *Cercaria hamata,* a furcocercous larva which was found experimentally to penetrate into the sunfish, where it developed into a larval holostome (McCoy, 1928). The definitive host is not known, but the cyclic fluctuations in the sea-

Data of Infestation with Larval Trematodes of Planorbis trivolvis *Collected from a Small Lake Near St. Louis, Mo.*

Date of Collection	No. of Snails	Species of Cercaria	No. of Infested Snails	Per Cent of Snails Infested	No. of Mature Infestations	Per Cent of Snails With Mature Infestations	Per Cent of Infestations Which Were Mature
10/ 5/25	68	Cercaria hamata	1	1.5	1	1.5	100
10/14/25	120	C. hamata	1	0.9	1	0.9	100
10/28/25	199	C. hamata	5	2.5	2	1.0	40
		C. of Plagiorchis ameiurensis	1	0.5	1	0.5	100
11/25/25	149	C. hamata	1	0.7	0	0.0	0
		C. of P. ameiurensis	1	0.7	1	0.7	100
12/ 9/25	76	C. hamata	7	9.2	5	6.6	71
		C. of P. ameiurensis	1	1.3	1	1.3	100
1/18/26	17	C. of P. ameiurensis	1	5.9	0	0.0	0
2/13/26	65	C. of P. ameiurensis	1	1.5	1	1.5	100
3/17/26	133		0	0.0	0	0.0	
3/24/26	143	C. hamata	2	1.4	0	0.0	0
4/19/26	207	C. hamata	3	1.5	0	0.0	0
5/13/26	234	C. hamata	7	3.0	7	3.0	100
		C. of P. ameiurensis	3	1.3	1	0.4	33
6/10/26	321	C. hamata	10	3.1	0	0.0	0
		C. of P. ameiurensis	1	0.3	0	0.0	100
8/30/26	275	C. hamata	10	3.7	8	2.9	80
		C. of P. ameiurensis	3	1.1	2	0.7	67
9/21/26	290	C. hamata	22	7.6	19	6.6	86
		C. of P. ameiurensis	10	3.5	2	0.7	20
10/13/26	197	C. hamata	19	9.6	3	1.5	16
		C. of P. ameiurensis	42	21.8	1	0.5	2
11/ 2/26	212	C. hamata	7	3.3	6	2.8	86
		C. of P. ameiurensis	69	32.5	16	7.5	23
11/26/23*	136	C. hamata	3	2.2	3	2.2	100
		C. of P. ameiurensis	9	6.6	4	2.9	44
12/10/26	214	C. hamata	11	5.1	0	0.0	0
		C. of P. ameiurensis	56	26.2	19	8.9	34
1/ 8/27	122	C. hamata	2	1.7	0	0.0	0
		C. of P. ameiurensis	21	17.8	7	5.7	33
1/31/27	72	C. hamata	3	4.2	0	0.0	0
		C. of P. ameiurensis	3	4.2	0	0.0	0
2/22/27	264	C. hamata	10	3.8	0	0.0	0
		C. of P. ameiurensis	77	29.2	28	10.7	38
3/24/27	189	C. hamata	11	5.8	0	0.0	0
		C. of P. ameiurensis	62	32.8	22	11.6	35
4/ 5/27	258	C. hamata	10	3.8	9	3.5	90
		C. of P. ameiurensis	78	30.2	62	21.7	80
5/26/27	387	C. hamata	16	4.2	16	4.2	100
		C. of P. ameiurensis	93	24.0	35	9.0	38
6/16/27	433	C. hamata	8	1.9	7	1.6	88
		C. of P. ameiurensis	43	10.0	11	2.5	25
7/ 6/27	412	C. hamata	18	4.3	12	2.9	67
		C. of P. ameiurensis	30	7.3	2	0.5	7
7/28/27	446	C. hamata	15	3.3	14	3.1	93
		C. of P. ameiurensis	46	10.2	15	3.3	33
8/17/27	444	C. hamata	23	5.2	23	5.2	100
		C. of P. ameiurensis	65	14.5	44	10.0	68
9/ 7/27	450	C. hamata	7	1.6	6	1.3	86
		C. of P. ameiurensis	20	4.4	5	1.1	25

* 75% of snails below average size; figures not included in curve.

sonal infestation of the snails with the cercariae indicate that it is most probably a water bird which is not present during the entire year.

Consideration of the seasonal cycle of this species will begin with the collection of June, 1926, in which none of the infestations were mature. During the summer and early fall, the percentage fluctuated but by December, 1926, all were mature (Figure 1, curve I). The figures for

the fall of 1925 are based on a smaller number of parasitized snails, but nevertheless, in general, verify this observation. None of the infestations found during the winter and early spring in 1926 and again in 1927 gave emerged cercariae, but they all had the appearance of being old, that is, the digestive gland, of the snail was completely permeated by sporocysts many of which were empty and partially collapsed. By the first of May practically all of the infestations found were mature.

The condition seems to indicate that ova are dropped into the water during the spring possibly by some aquatic bird. The coolness of the water probably retards the development of these ova and the first snails infected from them are not found until about the first of June, at which time the infestations are mostly immature. In no collection in the early summer of 1927 did all of the infested snails contain only immature cercariae as was the condition in June, 1926, but the distinct drop in the percentage of mature infestations in the collection of July 6 would indicate that a new infection had taken place in the late spring. The fact that the percentage of infested snails giving emerged cercariae did not drop very low in the early summer of 1927 might be explained by the spring infection occurring gradually. Infection probably occurs again during the late summer and early fall, but by the first of December nearly all infestations are mature. Apparently the sporocysts survive the winter in the snail, but no mature cercariae are given off again until about the first of May, when practically all infestations found are mature. These conclusions are based only on the evidence from the collection data, which may be influenced by such factors as the possible early death of parasitized snails, a low degree of infection of young snails, and so on. Complete understanding of the cycle of infestation can only be reached when the definitive host is discovered.

The second form to be considered is the cercaria of *Plagiorchis ameiurensis* which belongs to the group of Xiphidiocercariae and is the larva of a fluke parasitic in the intestine of the catfish (McCoy, 1928). Since the definitive host is present in the lake throughout the year, a continual occurrence of mature infestations might be expected in a temperate climate and such seems to be the case (Figure 1, curve IV). There are no clear-cut seasonal fluctuations in the infestation, but when the record as a whole is considered, great variation may be seen from time to time (Figure 1, curve V). Up until October, 1926, the percentage of infestation had been consistently very low, never exceeding 6%, but in the succeeding collections the percentage jumped to over 32%, falling irregularly during the course of the year, until in the fall of 1927 it was again below 10%. It is remarkable that in a seemingly balanced habitat, the occurrence of an established species should undergo such great variation, for reasons not apparent on the surface.

As a further observation on the instability of the larval trematode fauna of the lake, it may be noted that of the four other species of cercariae which were found in the study, but whose data of infestation are not included, one was present in only two collections during the first few months of the survey. Another species did not appear until the summer of 1927. The other two species were more or less irregular in their occurrence and always showed a very low percentage of infestation.

SUMMARY

The seasonal infestation with larval trematodes has been recorded for over 6,500 specimens of *Planorbis trivolvis* from a small lake near St. Louis, Missouri, over a period of two years. Although the data do not show any clearcut seasonal fluctuations, the degree of infestation varies widely from time to time.

From the curve of percentage of mature infestations of a furcocercous species, *Cercaria hamata* Miller, 1923, some inferences have been drawn as to the nature of the seasonal cycle. This larva is apparently able to survive the winter in the snail host.

Mature infestations of the cercaria of *Plagiorchis ameiurensis* McCoy, 1928, a fluke parasitic in the catfish, occur at all seasons of the year, a condition which, in a temperate climate, might be expected of a form whose definitive host is present in the lake throughout the year.

The exceedingly great variation in the percentage of infestation of the snails with the cercaria of *Plagiorchis ameiurensis* indicates that the larval trematode fauna of the lake is probably never in a very stable condition. Also the occasional appearance of new species and disappearance of old ones show a continual state of change, depending probably upon the visitations of the vertebrate hosts. The great variability in the degree of infestation makes isolated observations on this point of comparatively little value.

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NOTES ON LARVAL NEMAS FROM INSECTS

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Insects serve as intermediate hosts for a great many parasitic nemas. During the course of his investigations, the writer has repeatedly come across these larval forms, many of which undoubtedly pass their sexual stage in another host. It would seem that such observations are of sufficient interest to nematologists, especially to those working on life histories, to justify a permanent record. The writer therefore, proposes to publish from time to time, under the above title, a record of these findings together with as much information regarding the forms as can be readily obtained.

1. *Agamospirura melanopli* n. sp. from the Grasshopper, *Melanoplus femurrubrum*.*

While certain of the Blattidae are among the insects which commonly serve as intermediate hosts for the Spiruridae, grasshoppers appear not to be reported as serving in this capacity. During the past few years the writer has had the opportunity of examining several thousand grasshoppers collected from some eight or ten widely separated regions in the eastern and central United States. With the exception of the mermithids, both the Acrididae and the Tittigonidae have been found surprisingly free from nemic parasites.

During November, 1925, two larval Spirurids were taken from the body cavity of an adult *Melanoplus femurrubrum*. The infected insect was collected in an open pasture where grasshoppers were very abundant during the season of 1925. These parasites appeared not to have been encapsulated in the tissues but rather to have been free and active in the body cavity. When dissected out into salt solution they remained vigorously active for several hours.

The two specimens were of approximately the same size and gave the following measurements:

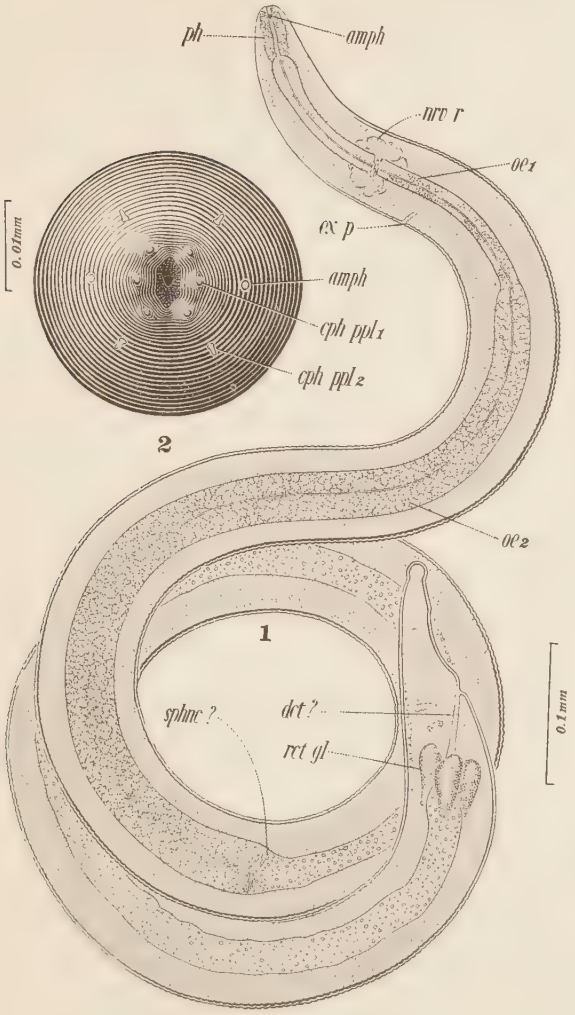
$$\frac{1.6 \quad 6. \quad 45. \quad \text{Juv.} \quad 95.}{1.6 \quad 2. \quad 3.2 \quad 3.5 \quad 2.2} 2.1 \text{ mm.}$$

The cuticula is about 2.8μ in thickness, colorless, and traversed throughout by moderately fine, uniform, transverse striae, not altered on the lateral fields. Longitudinal striation due to the attachment of the muscles is a particularly conspicuous feature, and with the possible exception of the extremities, can be seen over the entire surface of the body. At about the junction of the two portions of the esophagus

* Drawings prepared, under the writer's supervision, by Gertrude L. Power.

the cuticula shows a very slight, external, ridge-like thickening along each lateral line. These do not extend far either anteriad or posteriad and are the only indication of alae. Characteristic of many Spiruridae, the mouth is elongated dorso-ventrally, and when viewed from in front (Text fig. 2) is flanked on either side by a three-lobed lip. The lobes are rather closely amalgamated so that very little individual movement would be permitted, although each triplet can probably be moved somewhat as a unit. Each lobe bears on its anterior surface, a small, forward pointing papilla (*cph* ppl_1). Viewed laterally, the head is rounded to subtruncate in outline with the mouth opening very slightly depressed. The amphids (*amph*) are located back on the head about 10μ to 12μ from the circle of lip papillae. Their external manifestation is small and slightly oblong in outline, its greater diameter being perpendicular to the main axis of the body. When viewed in cephalic aspect, a tube-like connection can be traced posteriad a short distance where it appears to break up into a number of minute elements. Placed on about the same annule as the amphids, are four small, slender, cephalic papillae (*cph* ppl_2), two dorso-submedian and two ventro-submedian. The pharynx is simple, prismoidal and the lumen is about 8μ in diameter. It is not a conspicuous feature in either living or preserved material and was rather difficult to make out. The anterior portion of the neck is conoid, changing gradually to cylindroid posteriad. Lateral, cervical papillae which occur on some Spirurids, were not observed.

Characteristic of the group, the exceedingly long esophagus is divided into two parts (oe_1 and oe_2). The anterior portion is about 0.25 mm. long with an average diameter of 12 to 15μ, dilating to about 18μ at the anterior end. Its protoplasm is granular throughout, showing little evidence of muscular development. The posterior portion of the esophagus extends posteriad 0.68 mm. or nearly to the middle of the body. At its anterior end it has a diameter equal to about one-third the corresponding body diameter, increasing gradually until it is about one-half the corresponding body diameter. In appearance it is quite different from the anterior portion; its granulation is denser and of a coarse alveolar appearance in the living specimen. Both portions of the esophagus are traversed by a fine, strongly developed esophageal tube. There is little, if any interruption in the size or character of this tube at the point where the two portions of the esophagus join. At the junction of the esophagus and intestine there is a narrow band of what appears to be transverse muscle fibers suggesting a sort of sphincter muscle at this point (*sphnc?*). The intestine is rather irregular in outline and shows no well defined lumen. Its average diameter is about two-fifths the corresponding body diameter. It is rather sparsely filled with granules of varying shapes and sizes. The rectum is about 80μ in



TEXT FIGURE

1. Lateral aspects. 2. Cephalic aspect. (For details see text.)

length and at the point where it joins the intestine, is flanked by three conspicuous more or less ovoid rectal glands some 40μ long by 20μ in diameter. What may possibly have been ducts (*dct?*) extending from these glands and emptying into the rectum near the anus were observed but a careful study of this portion of the body left this point somewhat in doubt. The tail, although slightly asymmetrical, is conoid throughout and ends in a blunt, knob-like terminus armed with a few scattered and very inconspicuous projections of a spine-like nature (these projections not indicated in drawing). The excretory pore is located about 35μ behind the nerve ring, or back about one-third the distance from the nerve ring to the junction of the two portions of the esophagus.

THE INFECTIVITY AND PATHOGENICITY OF A STARCH-FED STRAIN OF *ENDAMOEBA HISTOLYTICA* *

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Dobell and Laidlaw (1926) reported that a strain of *Endamoeba histolytica* cultivated in Boeck and Drbohlav's medium which was enriched with rice starch, lost its pathogenicity. The tests were made on kittens beginning thirty-eight days after subculture in starch during which time thirteen transfers were made. Starch accelerated the growth of the amoebae—they were much more numerous than in the control tubes which were cultivated without starch. Eight kittens received rectal injections but none became infected, whereas a kitten, inoculated from one of the control tubes, promptly developed dysentery. Pathogenicity was not restored when the use of starch in the cultures was discontinued. The above data are of especial interest because, if corroborated, they will affect the claims of many protozoologists that strains of *E. histolytica* differing in virulence do not occur. The well known gradations of severity of human amoebiasis is explained by the above protozoologists with the statement that variations occur only in the resistance of the host. Kessel (1928) gives a good review of the literature on this question and supplies valuable data from his own experiments. In the infectious diseases on the other hand gradations of severity are attributed to an interplay of two variable forces (1) the pathogenicity of the etiological agent and (2) the resistance of the host.

From a study of the literature in amoebiasis it was noted that in only one investigation (Kruse and Pasquelle, 1894) was infection of kittens reported following rectal injection of trophozoites from human liver abscesses. Repeated failures of such experiments are reported by Wenyon (1912 and 1926) and others. The amoebae of these abscesses no doubt ingest liver tissue which is rich in glycogen (animal starch). It occurred to the writer that carbohydrate ingestion might render the amoebae more sluggish because of an accumulated food reserve. In that case they would not be so readily stimulated by the conditions of environment in the kittens' colon to establish themselves and would be voided. If such be the case Dobell and Laidlaw were in error in reporting a loss of pathogenicity. All that they demonstrated was an apparent loss of infectivity when the method of rectal injection was used without sub-

* From the Department of Protozoology of the Johns Hopkins University, School of Hygiene and Public Health. Aided by a grant from the Research Committee of the American Medical Association.

sequently closing the anus. It was obviously in order to test this assumption by a method of inoculation whereby escape of the parasites was prevented. Hence it was decided to use the laparotomy method, ligating the colon and injecting the amoebae through its walls. The details of this method are reported elsewhere (Rees, 1928).

The use of starch in the cultures was commenced on October 16, 1927, and the inoculation experiments October 19 (Table 1). The tests were continued until December 20 (63 days). At this time the starch strain was lost during the writer's absence from Baltimore. It was

TABLE 1.—*Data Obtained in Experiments with Kittens Injected (After Laparatomy) with Endamoeba histolytica Grown in Cultures Containing Starch*

Identification Number	Weight in Grams	Date	Number of Subculture	Amount Injected Cc.	Duration of Experiment, Hours	Manner of Death	Degree of Infection	Autopsy Record	
								Gross Lesions	Microscopic Lesions
1	600	Oct. 4	..	0*	44	Killed	—	None	Thickened submucosa
2	600	Oct. 19	2	3	140	Died	++	Slight	Necrosis penetration
3	600	Oct. 22	3	3	90	Killed	+++	Thickening, necrosis	Necrosis penetration
4	640	Oct. 26	4	3	27	Killed	+	None	None
5	585	Oct. 30	6	3	48	Killed	+++	Thickening	Slight necrosis
6	700	Nov. 3	8	3	36	Killed	—	None	Not sectioned
7	450	Nov. 3	8	1	27	Killed	—	None	Normal
8	400	Nov. 3	8	2	28	Killed	—	None	Normal
9	340	Nov. 3	8	1	65	Killed	—	None	Thickened submucosa
10	260	Nov. 3	8	3	42	Killed	—	None	Not sectioned
11	320	Nov. 6	9	5	90	Died	+++	Thickening, necrosis	Necrosis, little penetration
12	280	Nov. 6	9	5	66	Killed	+	Normal	Not sectioned
13	340	Nov. 6	9	5	18	Died	—	Normal	Not sectioned
14	800	Nov. 8	13	5	42	Killed	+++	Thickening, necrosis	Slight penetration
15	740	Nov. 8	13	3½	42	Killed	—	Normal	Not sectioned
16	920	Nov. 13	15	5	46	Killed	—	Normal	Not sectioned
17	800	Nov. 13	15	5	46	Killed	—	Normal	Not sectioned
49	400	Dec. 20	31	5	65	Died	++	Thickening, necrosis	Necrotic mucosa, thickened submucosa
50	400	Dec. 20	31	5	90	Killed	—	Normal	Normal
			no amoebae						

* Operated upon but injected with Locke's solution.

nearly lost on October 15, due to incubator trouble, which probably accounts in part for negative results in kittens 15, 16 and 17 though these animals were larger and past the age of greatest susceptibility to amoebiasis. It will be noted that of the first thirteen kittens inoculated seven became infected. In all infected animals killed after 40 hours the lesions were very severe. In numbers 2, 3 and 14 the mucosa was entirely necrotic, the necrotic tissue constituting a diphtheritic membrane. The submucosa was also invaded. The lesions are described in detail and figured in another paper (Rees, 1928). Unfortunately kitten 49, the only one inoculated after a period of subculture in starch comparable to the period of Dobell and Laidlaw's strain, died during the night. The

extent of necroses due to *E. histolytica* could not, therefore, be accurately determined because of autolytic changes. But the appearance was decidedly that of a dysenteric colon and many amoebae were still alive after 65 hours. Kittens 1 and 50 were controls. No. 50 was inoculated with 5 cc. of culture fluid containing the characteristic bacterial flora but no amoebae. The tissues of these colons save for slight inflammation in the serous and submucous coats, were perfectly normal. So also were the colons of 9 other kittens which did not become infected and which may, therefore, be considered as controls. No demonstrable attack on the tissues of the colon occurred under 40 hours and the evidence indicates that during at least 24 hours the organism is engaged in adjusting itself to the changed environmental conditions that occur in the kitten's colon. The experiments indicate that starch ingestion did not destroy the pathogenicity of the endamoeba.

As with Dobell and Laidlaw so in this work the amoebae grow prolifically in the starch containing media. After six months' experience with cultures of this amoeba, however, the writer has discontinued the use of starch as a means of maintaining laboratory strains. It appears to make the amoebae more sluggish; one to several intact starch grains can be detected in each individual organism. It is not needed as the organisms grow well without it. Furthermore its use adds factors to the already complex interactions between products of bacteria, yeasts and the endamoebae. The growth of starch splitting bacteria is especially favored and blastocystis seemed more common than in cultures without starch. As a means of "speeding up" a culture the day before use or when culture methods are used in fecal diagnosis starch may be profitably employed. In this laboratory both for starch and non-starch cultures incubator temperatures of 35° C. to 36° C. have given better results than higher temperatures.

CONCLUSIONS

These experiments indicate that the ingestion of starch by *Endamoeba histolytica* did not result in a loss of pathogenicity. The evidence also indicates that the tissues of the colon of the kitten are not attacked until the parasite adjusts itself to the new environmental conditions existing within the lumen. Also that the more sluggish starch fed amoebae may be ejected during that time unless the colon is ligated.

There appears to be a similarity in this respect between endamoebae that have ingested starch and those of liver abscesses that have ingested glycogen.

The disadvantages of the use of starch for the maintenance of laboratory strains of *E. histolytica* appear to outweigh the advantages.

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SOCIETY PROCEEDINGS

ABSTRACTS OF PAPERS CONTRIBUTED FOR THE FOURTH ANNUAL MEETING OF THE AMERICAN SOCIETY OF PARASITOLOGISTS, DECEMBER 27-31, 1928, NEW YORK CITY

Effect of Helminthiasis on Resistance of Chickens to Parasitism. James E. Ackert and Roy W. Jones, Kansas State Agricultural College.

Three experiments on 168 pure bred chickens were run to ascertain if a previous infestation with the fowl nematode, *Ascaridia lineata* (Schneider), would affect the resistance of the chickens to subsequent infestations with this worm. Half the number of chickens from a hatch were parasitized and this parasitism permitted to extend five weeks before these birds were again parasitized and the controls (other half of hatch) fed the same number of eggs. *Experiment I.* The 20 control chickens averaged 4.3 worms and the previously parasitized, 5.9 worms per bird; average length of worms of controls, 11.36 mm., previously parasitized, 11.26 mm. Resistance not significantly affected by the previous parasitism. *Experiment II.* The 20 controls averaged 5.85 worms and previously parasitized 1.68 worms, a significant difference indicating an increased resistance from the primary parasitism. The lengths of the worms was similarly 8.07 mm. for controls, and 5.67 mm. for previously parasitized. *Experiment III.* The 44 controls averaged 6.25 worms and the previously parasitized 8.47 worms. Difference not significant. Average length of worms of controls, 8.41 mm.; of previously parasitized, 4.97 mm. Difference not significant. Biometrical treatment of the combined data of the three experiments showed that the differences were within the limits of experimental error.

Changes in the Acid-Base Equilibrium of the Blood of Rats Infected with Trypanosoma equiperdum. Justin Andrews, Johns Hopkins University.

In twenty-eight rats, P_H determinations were made electrometrically with double quinhydrone electrodes at intervals of approximately thirty and seven hours before inoculation with *T. equiperdum* and at approximately eighteen, thirty-eight and forty-seven and a half hours after inoculation. On eleven of these animals determinations were obtained about seventy-two hours after inoculation, and on six others at about seventy-eight hours after. Determinations were made upon all the animals at the point of death. The average duration of the infection in this series of animals was 75.4 ± 0.57 hours. The means of all the P_H determinations made prior to the agonal syndrome varied from 7.615 ± 0.016 to 7.406 ± 0.032 and none of the averages varied significantly from the preinoculation determinations which were 7.603 ± 0.005 and 7.603 ± 0.002 respectively. The P_H determination made as the animal was clearly about to die was 7.205 ± 0.019. This figure is significantly lower than the preinoculation averages. These figures are uncorrected for dilution, temperature, and various other factors, and cannot in their present form be accepted as absolute determinations. It is concluded the rats infected with *T. equiperdum* die with a terminal acidosis.

The Mode of Action of Some Anthelminthics upon Ascaris. H. W. Brown, Vanderbilt University.

This study presents an attempt to ascertain the mechanism and the site of action, upon the pig ascarid, of some of the more common anthelminthics.

The Rates of Loss and Acquisition of Hookworms. Asa C. Chandler, Rice Institute.

The rapidity with which hookworms are lost and acquired under normal conditions is of great practical importance. The theory that hookworms are slowly acquired and slowly lost is based on unsound premises. There is a considerable mass of epidemiological evidence supporting the view that there is an annual replacement of a large percentage of the worms harbored. Some experimental evidence supporting this view was obtained in studies on inmates of Indian jails, after various periods of confinement. The evidence favors the view that 50% or more of the worms harbored are lost within six months, and about 70% in a year, but that there is a diminishing rate of loss after that for at least five or six years. If it is true that hookworms are rapidly lost and rapidly acquired, it would mean that improvement in the level of hookworm infection after sanitary reforms would follow rapidly, whereas anthelminthic treatment without measures to prevent reinfection would be followed by a more temporary lowering of the infection level, which has, in fact, been demonstrated. In countries with a long dry season, a cumulative increase in hookworms would be prevented; in such countries it would be of prime importance that mass treatments be given at the end of the dry season and not at the end of the wet season.

Observations on the Life History of the Swine Stomach Worm, Physocephalus sexalatus, *in the United States.* Eloise B. Cram, Bureau of Animal Industry, U. S. Dept. of Agriculture.

Larval nematodes found encysted in the walls of the digestive tract of loggerhead shrikes, screech owls, rats and snakes, as natural infections in abnormal hosts, were found to be identical with larvae from two dung beetles, *Phanaeus carnifex* and *Canthon laevis.* Experimental feedings of the larvae, both from the shrike and from the beetles, resulted in reencystment in the walls of the digestive tract of birds (hawks, owls, chickens, turkeys, quail and pigeons), mammals (skunks, opossums, guinea pigs, rats and mice) and reptiles (the black snake). Feeding to young pigs, however, resulted in the development of the larvae to adults, in the stomach, and the subsequent identification of the parasite as *Physocephalus sexalatus.* A restudy of spirurid larvae reported by Cram in 1924 as encysting in the pigeon, guinea pig and frog, the larvae having been collected from *Pinotus carolinus* in Washington, D. C., by N. A. Cobb, has resulted in the identification of these larvae also as *Physocephalus sexalatus.* These findings in the United States agree with Seurat's reports of encapsulated larvae of this species in various accidental hosts in Algeria. The three species of Coleoptera named above are reported here for the first time as intermediate hosts of this nematode. The only intermediate host previously reported in this country for *Physocephalus sexalatus* is *Onthophagus hecate* reported from Illinois by Raffensperger.

An Intestinal Infection of Ancylostoma braziliense *in a Boy and Skin Lesions Produced with Larvae from This Strain.* W. E. Dove, Johns Hopkins University.

In cultures of stool specimens from a boy in Texas a comparatively small number of hookworm larvae were obtained. It seemed likely that the larvae were the progeny of a single female worm. Some of them were used in inoculating "clean" kittens and adult hookworms were reared and recognized as *Ancylostoma braziliense.* Two lots of larvae obtained in cultures from the boy were allowed to penetrate the skin of a volunteer. As a check on these inoculations the stool specimens frequently were used in making charcoal cultures. From these cultures there was no evidence of the establishment of an intestinal infection of hookworms. It was evident that the boy harbored an intestinal infection of *A. braziliense,* and apparently other hookworms were not present. The writer was interested in the skin lesions produced by larvae from such a source. On two occasions, December 4, 1927, and March 24, 1928, larvae were obtained in cultures from the boy and were

allowed to penetrate the skin of a volunteer. Following each application there was a diffused erythema and a marked swelling of the forearm. Within three days the swelling subsided leaving papules somewhat smaller than a pea. In association with the papules there were numerous vesicles about the size of a pinhead. These were in rows of less than two cm. in length, and presented a linear lesion with a beaded appearance. Following the third day there was apparently no further migration of the larvae. The pruritic symptoms were present for a period of slightly more than a week, after which the affection disappeared without treatment. Some larvae obtained in cultures from the boy were carried through a single generation in a "clean" kitten, and the progeny were allowed to penetrate human skin. In less than four days linear lesions of creeping eruption were produced. Two lesions of this infection were active for more than 30 days.

Crepidobothrium fragile, *a New Species of Tapeworm of the Channel-Cat* (Ictalurus punctatus). Hiram E. Essex, Mayo Clinic.

From examinations of the intestinal parasites of 180 channel-catfish from Rock River (Illinois) only 15 tapeworms were collected that could not be referred to the genus Corallobothrium. These unusual cestodes constitute a new species which belongs to the genus Crepidobothrium. Because of the fragile nature of the strobila it has been called *Crepidobothrium fragile.* This is the first recorded instance of a species of that genus infesting a fish host. They are commonly found in reptiles and amphibians. The majority of the 15 specimens obtained were sexually mature. Therefore it seems rather improbable that their presence in the channel-catfish represents an accidental infection with a species that is normally parasitic in some other host. If such were the case this tapeworm would not be expected to follow a normal course of development.

An Interesting Cestode Larva from the Liver of the Common Bullhead (Amieurus nebulosus). Hiram E. Essex, Mayo Clinic.

From the livers of five *Amieurus nebulosus* taken from the Mississippi River near Homer, Minnesota, 8 cysts were obtained which measured about 0.77 by 0.66 mm. Each contained a larval cestode that possessed 4 protrusile proboscides without hooks or spines and, so far as could be determined, without accessory bothria or acetabula. The character of the scolex is suggestive of the Trypanorhynchids the adults of which are found exclusively in Elasmobranch fishes. However, the absence of hooks precludes its being placed in that order. Up to the present I have not found a place in the existing classification for this interesting form. All efforts to connect this larval cestode with any adult form have proved ineffectual as it bears no resemblance to any of the tapeworms described from fresh water hosts.

Morphological and Experimental Studies on Species of Diphyllobothrium in the Far East. Ernest Carroll Faust, Tulane University. (Invited Paper.)

From the studies of the genus Diphyllobothrium by the writer and his associates during the last few years the following facts have developed. Four well defined species of Diphyllobothrium (*D. mansoni, D. decipiens, D. ranarum,* and *D. erinacei*) with spargana in a variety of vertebrate hosts (frogs, snakes, mammals), develop as adults in cats or dogs but fail to develop experimentally in man. Another species, taken by Dr. H. S. Houghton from the intestinal tract of man in Shanghai, as well as by the writer from dogs and cats in China, differs specifically from these other forms and will be separately named and described. Hatched embryos of *Diphyllobothrium decipiens* and *D. erinacei* have been found to penetrate and develop into mature procercoids in the body cavity of a large number of *Cyclops* in the Peking area. Ocular sparganosis has been produced experimentally in dogs by applying living spargana to the conjunctiva. Cases of human sub-

cutaneous sparganosis in Fukien Province, South China, give a history of having applied fresh frog flesh to an ulcer or felon, in a manner similar to that practiced by the natives of French Indo-China suffering from ocular sparganosis (Joyeux and Houdemer). It seems likely, therefore, that human sparganosis results primarily, although not necessarily exclusively, from the application of fresh animal poultices infested with spargana, presumably of any of the species utilizing such animals as secondary intermediate hosts.

The Incidence of Malaria in South China in Relation to Humidity and Temperature. Ernest Hartman, Lingnan University.

An examination of the cases of clinical malaria occurring in the vicinity of Canton, China, and of local meterological data is being made to determine the relation between weather and incidence of malaria. Accurate daily weather records have been kept for the past nine years. The average of three daily readings of the relative humidity seldom falls below 50%. The maximum yearly temperature is seldom above 35°C. and very seldom below 2°C. Thus the weather conditions are not extremely unfavorable for mosquitoes at any time of the year. Clinical malaria occurs throughout the year with the peak occurring in the months of September, October and November which is part of the "dry" season. From the data available it appears that in this region the optimum condition for the mosquito transmission of malaria is an average relative humidity of 70-80% together with a temperature between 18°C. and 28°C. The greatest number of malaria cases does not occur during the heaviest rainy season.

Transmission of Intestinal Protozoa from Man and Other Animals to Parasite-Free Fowls. Robert Hegner, Johns Hopkins University. (Invited Paper.)

Fowls were used for these experiments because they could be obtained parasite-free from the egg and could be maintained parasite-free with very little difficulty. Twenty-one species of intestinal protozoa from man and other animals were inoculated either per rectum or per os into parasite-free chicks from 3 to 45 days old. Some of the results obtained are as follows: Protozoa inoculated per os may reach the ceca in a viable condition within one hour. Infections with foreign protozoa were all localized in the ceca. With the exception of *Giardia lamblia* from man, *G. muris* from the rat and *Endolimax nana* from man none of the protozoan cysts injected into the chicks excysted. Infections were established in chicks with a number of the protozoa used, especially with trichomonads. Flagellates of the genus Trichomonas obtained from the mouth and intestine of man, intestine of monkey, cecum of rat and intestine of prairie dog were recovered at intervals ranging up to from 107 to 191 days, indicating that what may be called permanent infections had been established. Parasite-free fowls became infected by association when placed in the same cage with fowls parasitized by these foreign protozoa. There appears to be no doubt but that fowls are capable of transmitting certain human intestinal protozoa. The fact that fowls may be infected with trichomonads from at least seven different species of hosts suggests that at least several of these trichomonads may belong to a single species and not to separate species as regarded by many at the present time.

The Life History and Control of Dactylogyrus sp. Walter N. Hess, Hamilton College.

This fluke was found abundant on the gills of the small mouth and large mouth black bass, common sunfish, goldfish, carp and other fishes. As many as 1,600 flukes were taken from the gills of a fish 11 inches long, and 47 from a fish three-fourths of an inch long. Serious losses occurred in hatcheries among fry between three and six weeks of age. During warm weather an average of about eight eggs are laid daily, which instead of being attached to the gills of the fish are discharged into the water. At this season the eggs hatch in about three days into ciliated larvae,

which, provided no fish are present, die in from four to six days. If, however, a fish is found, the larvae immediately attach, and undergo metamorphosis to the adult form in a few seconds. During cold weather (March and April) experiments show that about three weeks elapse between the laying of the eggs and the end of the larvae period. Some evidence is available to show that Dactylogyrus is not only a serious pest in hatcheries but also may cause considerable losses among fry of wild fishes with nest building habits. Methods for the control of Dactylogyrus on fishes, both in ponds and in small tanks were worked out, these methods proving very satisfactory on a large commercial scale.

The Ring Test in Fasciola hepatica *Infection of Cattle.* W. A. Hoffman and Trinita Rivera, School of Tropical Medicine, University of Porto Rico.

Experiments carried on thus far with the immunological method known as the "Ring Test" or "Precipitin Test," indicate its superiority to fecal examination for the diagnosis of *Fasciola hepatica* infection in cattle.

Notes on the Phyllodistomes of North America. Fred J. Holl, University of Buffalo.

Osborn (1903), Stafford (1904), and Pearse (1924) described new species of phyllodistomes from the urinary bladders of salamanders and fishes. While examining fishes in North Carolina two species were found; one, in the yellow bullhead, *Ameiurus natalis* (Le Sueur); and the other, in the blue-spotted sunfish, *Enneacanthus glorious* (Holbrook). This genus appears to have its species limited to certain drainage systems, since *Phyllodistomum superbum* Stafford has only been reported from the St. Lawrence drainage and *P. fausti* Pearse from the Mississippi, although *P. staffordi* Pearse is found in both of the above mentioned systems. The two new species were found in the South Atlantic watershed and are similar to those species found in the other localities. It is possible that this genus has its center of distribution in the waters of the South Atlantic states.

Life History Studies on Proteocephalus pinguis La Rue. George W. Hunter, III, Rensselaer Polytechnic Institute.

During the summer of 1928 while doing some work for the New York State Conservation Commission it was possible to secure various developmental stages of *Proteocephalus pinguis* La Rue. The adult occurs in *Esox lucius* and *E. reticulatus.* The eggs were eaten by two species of Copepoda, *Cyclops vulgaris* (=*C. viridis*) and *Leptocyclops agilis* (=*C. serrulatus*). These were infected experimentally in 81% and 50% of the cases respectively. These two forms may also harbor the procercoids of *P. ambloplitis.* Negative results were obtained with *C. annulicornis* (=*C. albidus*) and *Daphnia pulex.* The fish experimentally infected by feeding Copepoda containing the procercoids are, *Perca flavescens* (100%) and *Notropis atherinoides* (50%). Negative results were secured with *Eucalia inconstans.* The infected fish were in turn fed to various species of Esox and the advanced plerocercoids were recovered in 50% of the cases. Controls of copepods and fish were examined.

Further Experimental Studies on the Bass Tapeworm, Protocephalus ambloplitis (Leidy). George W. Hunter, III, and Wanda Sanborn Hunter, Rensselaer Polytechnic Institute.

During the summer of 1928 experiments were carried on for the New York State Conservation Commission to determine the number and variety of intermediate hosts of *Proteocephalus ambloplitis* (Leidy). Eggs were fed successfully to both *Cyclops vulgaris* (=*C. viridis*) and *Leptocyclops agilis* (=*C. serrulatus*) and yielded 50% and 25% infection respectively. Negative results were secured with *C. annulicornis* (=*C. albidus*) and *Paphnia pulex.* The following species of fish

were artificially infected by feeding infected copepods, *Ambloplitis rupestris* (80%), *Perca flavescens* (66%), *Fundulus diaphanus* (50%). Negative results were obtained with *Notropis hunsonius, N. atherinoides* and *Hyborhynchus notatus.* Infection was transferred by feeding infected liver to yearling *M. dolomieu* and *A. rupestris.* Developing plerocercoids were also recovered from the digestive tract of yearling *M. dolomieu* by feeding them infected *E. gibbosus.* Examinations of fish from a heavily infected pond showed that *E. gibbosus, P. flavescens* and *Esox reticulatus* may all carry the larval stage of *P. ambloplitis* in the body cavity. Controls of Entomostraca and fish were made.

The Protozoa of the Human Mouth. Charles A. Kofoid, University of California. (Retiring Presidential Address.)

This is a summary of the protozoological work of the Stomatological Group working under a grant of the Carnegie Foundation at the University of California under the direction of the writer in the past four years. The purpose of this investigation has been the scientific study of pyorrhea. These studies have demonstrated that *Endamoeba gingivalis* is generally present in the pus pockets of pyorrhea from the incipient stages throughout the continuance of the disease. The amoebae may be detected either by direct smear or by culture methods. In cultures from some cases in which amoebae are clearly demonstrable in smears no amoebae developed *in vitro,* presumably because of the presence of bacteria detrimental to their growth in culture media. Quite frequently amoebae are demonstrable by the culture method even where intensive smear examination has failed to reveal their presence. *Endamoeba gingivalis* occurs in most, if not all, cases of incipient to advanced pyorrhea of the inflammatory and advanced type. *Trichomonas buccalis* is found (in San Quentin Prison) in a high percentage (86%) of the cases of advanced pyorrhea along with *Endamoeba gingivalis.* It has not been found in normal mouths. These protozoan infections are often closely restricted to limited areas in the subgingival groove. The distribution of *Endamoeba gingivalis* in pyorrheal pockets is strikingly limited. In a typical case of pyorrhea with serumal calculus we found the calculus deposited around the bases of the filamentous bacteria, Leptothrix and Actinomyces. The surface of the tartar is covered by the felt of projecting free filaments in the midst of which is an almost continuous mass of motile amoebae. Their numbers are greatest at the point of greatest elevation of tartar. They continue down the deeper slope of the tartar to the bottom of the pocket a little in advance of tartar formation. They have not been found invading the adjacent tissues of the gingival groove. Immediately adjacent to this complex of bacteria and amoebae is the pus. Only 5% of the amoebae are found intermingled with the pus. The amoebae feed upon the denuded nuclei of the pus cells and have been found actively stripping the cytoplasm from the nuclei. As many as thirty-five leucocytic nuclei have been observed in a single amoeba. Chromatin in the food vacuoles is liquefied and is frequently extruded in long viscous threads. This seems to be a normal process by means of which a part at least of the phosphorus-rich nucleo-proteins are released in the immediate neighborhood of the deposition of tartar. In no case have amoebae been found actively penetrating normal or even diseased tissues of the gingivae adjacent to the pyorrheal pocket. The picture presented by this complex can be seen only in carefully preserved material of tissue of the teeth and alveolar sockets removed intact, so as to preserve throughout decalcification the normal relations of tartar, bacteria, amoebae, leucocytes and the gingival and alveolar tissues. Resorption of bone at levels below that of the pus pocket is apparent, but amoebae are not present in these areas. This complex may be interpreted as a symbiosis of amoebae and bacteria, the latter being active agents in tartar deposition, the amoebae contributing to the liberation of the phosphorus compounds from the nuclei of leucocytes and the alveolar socket contributing the calcium salts utilized in the deposition of tartar. The possible toxic action of bacteria or amoebae upon adjacent tissues with resulting chemotropic accumulation of leucocytes is suggested by this pathological picture.

The Life History of Ophiotaenia testudo Magath. Thomas B. Magath, Mayo Clinic.

A study of the life history of this parasite of the soft shelled turtle has been made. It follows, during its early life history, the same general scheme described for other proteocephalids. Its first intermediate host was found to be a cyclops.

Further Studies on Species of Chara and other Aquatic Plants in Relation to Mosquito Breeding. Robert Matheson and E. H. Hinman, Cornell University. (Invited Paper.)

The study of the effects of *Chara fragilis* on mosquito development reported in 1928 has been continued and expanded during the past season. Our investigations were directed along five principal lines: (a) continued studies of the effects of *Chara fragilis* on larval development under experimental conditions; (b) a survey of a portion of the central New York area to determine the distribution of the different species of Chara and its effect on mosquito density; (c) the introduction of *Chara fragilis* into various types of ponds, marshes, etc., to study its rate of growth and effect on future mosquito breeding; (d) a continued study of the food organisms (plancton) in Chara ponds and typical mosquito breeding pools; (e) the effect of other aquatic plants on the larvae of Culicidae. The studies to be reported confirm to a marked degree the main results obtained under experimental conditions during the season of 1927. Our survey and introductions open up new lines of research. The other plants to be reported on were tried in a limited way under experimental conditions.

Two New Marine Trematode Life Histories. O. R. McCoy, Johns Hopkins University.

Cercaria floridensis n. sp. from the marine snail *Cerithium litteratum* at Tortugas, Florida, has been identified as the larva of a member of the genus Acanthochasmus Looss. This larva is a lophocercous "monostome" cercaria characterized by the presence of eye spots, seven pairs of penetration glands and a dorso-ventral fin-fold on the tail. The cercariae were found experimentally to encyst in the fins and underneath the scales of small fish. Most of the adult characters then develop in the metacercaria; a ventral sucker is formed, the digestive tract develops and most striking a single row of 27 large spines appears in a complete circle around the mouth. Although the adult has not yet been determined, the cercaria can almost certainly be assigned to the genus Acanthochasmus. This observation throws light upon the type of life history to be expected of several described species of cercariae very similar to *C. floridensis* which heretofore have been grouped under the monostomes. The second life history is that of a cotylocercous cercaria from *Astraea americana* which encysts in the tissues of small fish. Fish experimentally infested with these cysts were fed to the gray snapper, *Neomaenis griseus*, and adult worms developed in the intestine and pyloric ceca which were identified as *Hamacreadium mutabile* Linton, 1910, a member of the sub-family Allocreadiinae. The cotylocercous or "stumpy-tailed" cercariae form a well-defined group of marine cercariae representatives of which have been described from several widely separated parts of the world. Heretofore no life history of any member of the group has been known.

The Growth of Hookworm Larvae on Pure Cultures of Bacteria. O. R. McCoy, Johns Hopkins University.

Ova of the dog hookworm, *Ancylostoma caninum*, have been obtained in large quantities free from fecal material by washing the feces of a heavily infested dog through a series of fine sieves. The filtrate was then sedimented, the sediment thoroughly washed, and the ova floated free in saturated salt solution. Ova obtained by this method were sterilized by treating for 30 minutes with a 5% antiformin solution in 10% formalin. These sterile ova are inoculated onto agar

cultures of bacteria in Erlenmeyer flasks; the ova hatched normally and the larvae grew to the infective stage in the usual period of about 7 days. When ova were put on sterile agar, the larvae which hatched lived for as long as 10 days but did not grow. Larvae have been raised so far on agar cultures of unidentified fecal bacteria and pure cultures of *Bacillus coli* and *Staphylococcus aureus*. The larvae have also been raised to the infective stage in heavy suspensions of *B. coli* in normal salt solution using both living and dead bacteria. This study demonstrates that hookworm larvae are able to utilize bacteria as their sole source of food.

Parasites and the Aid They Give in Problems of Taxonomy, Geographical Distribution and Paleogeography. Maynard M. Metcalf, Johns Hopkins University. (Invited Paper.)

A discussion is given of the use which has been made of data from parasites in studying problems of taxonomy, geographical distribution and paleogeography. Nine men have made use of such data in these broader ways: H. von Ihering, Vernon Kellogg, H. U. Williams, Launcelot Harrison, S. J. Johnston, Maynard M. Metcalf, S. J. Darling, H. E. Ewing and R. W. Hegner. The work of all of these men is reviewed to illustrate and emphasize the significance of data from parasites and from hosts when studied together. Parasites in common may indicate common ancestry for different hosts. For example, the biting lice of the penguins and of pigeons, tinamous and fowls indicate common ancestry for these hosts. Again, the common ancestry of the "southern frogs" of Australia and South America has been doubted, but their possession in common of an exclusively Southern Hemisphere species of parasite, Zelleriella, removes this doubt. The origin and route of dispersal of an organism may be indicated by its parasites. For example, the "southern frogs" are found only in Tropical America and in Australasia. It has been claimed that they arose in Arctogeaea and passed southward independently to tropical America and Australasia. This claim is refuted by the fact that neither southern frogs no Zelleriella now occur in the Northern Hemisphere outside the tropics, although Zelleriella passes readily from "southern frogs" to toads, Bufa and to some other genera abundant in North America and Euroasia. Not only may the absence of its parasites be an indication that an animal did not formerly inhabit a certain area, but the presence of the parasite may indicate the former presence of its host. For example, the presence in Australia of members of the sub-genus of Opalinids characteristic of the bell-toads indicates the former presence of bell-toads in Australia, probably the ancestors of Liopelma which migrated across Australia to New Zealand. Finally, the presence in widely separated areas of related animals with common or closely related parasites may indicate former communication between the now distinct regions. Such considerations open many questions of inter-continental connections in former geologic periods. In all study of biogeographical data, parasites as well as hosts, should if possible be taken into account. This has not been done at all for plants and has never adequately been done except for a very few groups of animals. Conspicuous examples of the crucial nature of the evidence from parasites will be shown.

Possible Rôles of the Behavior of Cercariae in the Life Histories of Trematodes. Harry M. Miller, Jr., Washington University.

The behavior of cercariae emerged from the mollusk host has been studied in some degree of detail for more than 40 freshwater and marine species, and its specificity and wide variety noted. The reactions to light, particularly to changes of light intensity, have been most varied; these have been studied under conditions simulating natural ones and under strictly experimental conditions in the darkroom. Some of the possible rôles which the various types of behavior may play in the life histories will be briefly discussed. When it is possible to take into account the relation between the type of behavior of the cercaria and the habits of its intermediate hosts the value of these reactions in furthering the life history will be more clearly understood.

Observations on the Development of Ova of Ascaris lumbricoides *in Various Concentrations of Atmospheric Moisture, with a Note on Viability in Soil Cultures.* G. F. Otto, Johns Hopkins University.

Ova of pig ascaris in the unsegmented and the first cleavage stages were exposed on glass slides in air having a known moisture content. At room temperature in a moving atmosphere 80-85% saturated with moisture development was slower than in water cultures or in a saturated atmosphere but at the end of 30 days less than 20% of the ova were definitely dead while 57% contained active embryos. In a saturated atmosphere over 80% had active embryo while 11% were dead. At room temperature in a greatly varying relative humidity which averaged about 70% practically all the ova were killed before attaining complete development. Only 3% of the ova cultured in water after being 30 days in this atmosphere developed motile embryos. Exposure at room temperature in an atmosphere less than 50% saturated for four days seemed to be lethal. A saturated atmosphere at 30°C. was unfavorable and only a few embryonated ova were obtained from three experiments though the controls in water at 30°C. developed normally each time. Human ascaris ova in feces were planted on barren level spots in the sun in the mountains of southwestern Virginia on July 14. On cinders which dried rapidly and reached temperatures above 42°C. only an occasional egg developed while on clay and sandy soils 26 and 38% of the ova isolated on the 83d day still contained coiled embryos.

Studies on the Survival of the Ova of Toxocara canis *Under Natural Soil Condition.* William B. Owen, University of Minnesota.

Studies made in the vicinity of Minneapolis, Minn., indicate that approximately 80% of the ova of *Toxocara canis* survived the winter months under natural soil conditions. Records of soil temperature, air temperature and precipitation are given with these data. Ova under natural soil conditions and exposed to full sunlight did not survive the summer months in the vicinity of Minneapolis. In the western part of Kentucky ova exposed in various types of soil to full sunlight did not survive the summer months while ova exposed in similar types of soil but in shade did survive. Records of soil temperature, air temperature and precipitation are given with these studies. Some studies on survival of low temperatures under laboratory conditions are included.

Mites from Land Crabs. A. S. Pearse, Duke University.

At Dry Tortugas, Florida, three species of mites were found on the gills of the land hermit crab, *Cenobita diogenes* (Latreille), and the Nassau crab, *Gecarcinus lateralis* (Freminville). One of these is the type of a new family, genus (Ewingia), and species. It has the two posterior pairs of legs modified into chelate clasping organs which somewhat resemble the legs of certain lice.

The Numbers of Trichomonas muris *in Relation to the* P_H *and Bacteria in the Cecum of Rats on Diets of Different Protein Content.* Herbert Ratcliffe, Johns Hopkins University.

Observations on cecal material from 68 rats fed diets containing 20 to 30% protein have indicated (1) that the number of *Trichomonas muris* per unit volume of cecal contents is inversely proportional to the number of proteolytic anaërobes present, and (2) that, in general, the larger numbers of trichomonads are associated with larger numbers of the other types of bacteria present and a slight acidity of the cecal contents. In order to investigate these relations further, three other diets were planned to contain 54% protein from (1) dried beef liver, (2) dried beef steak and (3) casein, together with 6% protein from dried yeast and salts and other substances necessary for a complete diet. Ten rats were used with each of these diets and observations were made at 10 day intervals on material obtained by surgical methods from the ceca of these animals. The effects of these

diets upon cecal conditions were as follows: The liver-yeast diet caused a drop in the P_H, and in the number of proteolytic anaërobes and aciduric organisms, while the numbers of *T. muris* increased greatly, accompanied by a large increase in the number of fermentative anaërobes and coli-aerogenes bacteria. The steak-yeast diet did not appreciably effect the P_H but caused a very definite decrease in the number of *T. muris* and aciduric organisms, while the other types of bacteria increased in numbers. The casein-yeast diet produced an alkaline condition in the cecum and caused a very rapid decrease in the number of *T. muris* and aciduric bacteria, while the fermentative anaërobes and coli-aerogenes bacteria were not appreciably changed, and the proteolytic anaërobes increased in numbers.

Nuclear Division in Hydramoeba hydroxena. Bruce D. Reynolds and Wm. L. Threlkeld, University of Virginia.

In this amoeba division is accomplished by a peculiar type of promitosis; there is also a distinct periodocity associated with this phenomenon.

The Incidence of Protozoal Infections of Man in Minnesota. William A. Riley, University of Minnesota.

An analysis has been made of a detailed study of 200 cases from Minnesota positive to protozoal infections. The results indicate wide distribution of six species of intestinal Amoeba, Chilomastix and Giardia. The incidence of *E. histolytica* is much lower than has been indicated by some recent reports.

American Anopheline Mosquitoes in Relation to the Transmission of Malaria. Francis Metcalf Root, Johns Hopkins University. (Invited Paper.)

A survey of the evidence available on the relative importance of the various American species of *Anopheles* as vectors of malaria indicates that out of over forty valid species, only about six are probably malaria transmitters of real sanitary importance. These six species are:

A. quadrimaculatus Say—Eastern and Southern United States.

A. maculipennis Meigen—Pacific coast of United States.

A. albimanus Wiedemann—Greater Antilles, Central America, Colombia, Venezuela, Ecuador.

A. tarsimaculatus Goeldi—Lesser Antilles, South America, Panama.

A. albitarsis Arribalzaga—Venezuela, Guiana, Brazil, northeastern Argentina.

A. pseudopunctipennis Theobald—northwestern Argentina.

In view of the desirability of keeping the cost of malaria-control campaigns as low as possible, emphasis is laid on the fact that in such campaigns anti-anopheline measures should be concentrated, so far as is possible, on the species which are really important carriers of the disease. It is pointed out that the breeding-places of these species are often different enough from those of the harmless species so that "species control" or at least "group control" is entirely feasible in practice.

Preliminary Report of Life-History Studies on a Rare Human Parasite, with the Demonstration of the Ova. J. H. Sandground, Harvard University.

The parasite under investigation was harbored by a white medical missionary who had spent about 20 years in Southern Rhodesia and Portuguese East Africa. The eggs are 72 to 103μ in length by 37 to 45.5μ in breadth, and were previously diagnosed as "hookworm." In addition to the shape and size of the egg, a differentiation from "hookworm" may be made on the basis of the morphology of the rhabditiform and filariform larvae, and also on the behavior of the later. Infection is contracted by the oral route. A human infection matured in about 7 weeks. Treatment with (1) carbon tetrachloride, (2) tetrachlorethylene, has failed to dislodge the parasites. Experimental infections of dogs have been unsatisfactory. In the region in which the original patient probably contracted infection, 82% of the stools are stated to contain hookworm eggs. Eight worms secured from treat-

ment of a native in Southern Rhodesia and previously regarded as *Necator americanus* were found to be *Ternidens deminutus*. This parasite, which probably lives in cysts in the large intestine, has hitherto been reported on two occasions from man. Evidence available indicates that it is more prevalent in man in certain parts of Africa than present knowledge shows.

The Specificity of the Parasite for the Host in Its Relation to the Problems of Age Resistance and Acquired Immunity with Special Reference to the Helminthiases and Other Metazoan Infections. J. H. Sandground, Harvard University

The occurrence of resistance to helminth infections attributable to the age of the host is more circumscribed than is generally believed. It has thus far been demonstrated to apply to the chicken in infections of Ascaridia and Syngamus to the dog in infections with *Ancylostoma duodenale* and *A. caninum*. It does not apply to infections of the turkey with Syngamus and man with *A. duodenale* or *Ascaris lumbricoides*. Resistance to infection with Toxocara in the dog and with Ascaris in the pig is probably due to previous infection. Immunity has been demonstrated to be acquired by dogs and cats to Strongyloides, by dogs to Nanophyes, in both of which instances the immunity is correlated with abnormality in the specific host-parasite association. The claim for acquired immunity in the rat against trichinosis should be confirmed and further investigations are necessary to establish the type of resistance to ascariasis in the pig and to *Hymenolepis nana* in the rat. The guinea pig, in contradistinction to autochthonous hosts like the rat, rabbit and dog, acquires immunity to *Chodylobia anthropophaga* (Diptera). Consideration of the nature of specificity indicates that it is a reflection of the extent to which the parasite is adapted to the host and is a product of an evolutionary process. The high correlation of resistance with an abnormality in the specific host-parasite association is significant, and permits of an understanding of the nature of the non-induced age-resistance. Similar considerations afford a clue for further work on the mechanism of acquired immunity in particular instances.

A Study of the Length of Life of the Dog Hookworm, Ancylostoma caninum. Merritt P. Sarles, Johns Hopkins University.

Laboratory bred litters of pups, 9 to 11 weeks old, were infected by oral administration of 500 larvae of *A. caninum,* in gelatin capsules. The resulting infestations were studied by dilution egg counts of the hookworm ova found in the total feces passed for three-consecutive-day periods. Salt flotation diagnosis was used to study the initial and final egg production. The first eggs appeared 14 to 16 days after infection. The average daily egg output increased to a peak of egg production which occurred from 3 to 17 weeks after infection. The egg production then fell off at a constant rate becoming negative to egg count 44 to 83 weeks after infection. In these pups a large percentage of the larvae given developed to maturity, and 94 to 97% of the worms were lost after 6 months, but a few remained as long as 21 months. After the dogs had lost their first infection they were re-infected. These infections of the adult dogs differed from their previous infections when pups by being of shorter duration, 0 to 34 weeks, and of small size, a few or no worms developing. Comparison of these re-infected dogs with a previously uninfected control adult indicate that most, and possibly all, of the increased resistance of the adult dogs to hookworm infection is due to an age resistance and not to an acquired immunity.

Observations on the Life History of Stephanurus dentatus. Benjamin Schwartz and E. W. Price, Bureau of Animal Industry, U. S. Department of Agriculture.

Under ordinary laboratory conditions the eggs of the so-called "kidney worm" of swine, *Stephanurus dentatus,* hatch in about two days and the larvae, after undergoing two molts in the course of about three or four days, reach the infective stage. The larvae do not appear to be capable of penetrating the intact skin of

swine; when placed on the scarified skin, on the nasal mucosa, injected subcutaneously or administered by mouth, infection takes place readily, the course of development from the time of the experimental administration of the larvae to the time when the worms are sexually mature in the peri-renal fat and in the ureters apparently requiring about five months or longer. Apparently irrespective of the portal of entry into the body, the larvae reach the portal vein and the gastro-hepatic artery, in which vessels they occur in thrombi. Immature worms occur in various visceral organs, notably in the liver, lungs and pancreas, the liver being most heavily and invariably parasitized. Immature worms have also been found in thrombi in the posterior venacava from which their path to the lungs can be readily understood. The young worms come gradually to the surface of the liver, lying underneath the capsule. In the course of time some of them penetrate the capsule to the exterior, thus liberating themselves from a location in which many of them perish and degenerate. Migration from the liver to the kidney fat is probably an active one, the distance to be traversed being comparatively small. The means by which fistulous channels are established between the ureters and the pus pockets in which the worms lie, are not yet ascertained.

Physiological Strains of Ancylostoma caninum. J. Allen Scott, Johns Hopkins University.

Two types of physiological strains of the dog hookworm, *Ancylostoma caninum,* which are adapted to life in the dog and in the cat respectively have been discovered in these two hosts. Although they are morphologically identical these strains differ in their ability to become established in these hosts. A strain designated at D 0 has been used for experiments reported previously. In a series of puppies, litter mates of which were used at the same time for other strains discussed here, an average of 46% of the larvae matured. In a series of kittens of the same relative age an average of only 5% of larvae from the same cultures matured. Another strain designated as C 1 originally found in a cat from Long Island has apparently become adapted to the cat. In a series of kittens, litter mates of those mentioned above and inoculated at the same time, an average of 45% of the larvae of this strain matured, while in puppies an average of less than 1% matures. Experiments show that when the strain D 0 had been in a cat one generation, the resulting larvae had not become more infective to the cat nor less so to the dog. Evidence now being collected indicates that in localities where the dog hookworm is common in cats, a host strain is present in these animals which is similar to the above strain C 1. This conclusion is based on the fact that several such strains have been tested experimentally. These facts suggest that this adaptation to life in the cat has been relatively recent, has occurred several times, and is relatively unstable.

The Growth in Length of Several Strains of Hookworms in Dogs and Cats. J. Allen Scott, Johns Hopkins University.

A photographic method has made possible relatively rapid measurement of the length of adult hookworms, *Ancylostoma caninum,* of various ages from both cats and dogs. Growth curves of the logistic type have been fitted to the data. Sufficient data concerning a dog strain in dogs are available to demonstrate that the growth of this metazoan parasite follows the typical course of growth determined for other biological forms, A study of the variation in the length of the worms and the variation in the average length of all the worms from various hosts has not revealed any factor having a pronounced effect on either of these types of variation. Age of host, size of the infestation, and the genetic histories of the host and parasite were some of the factors tested. The species of host had, however, a noticeable effect on the growth of the parasites. Growth was slower and the final size attained was less in cats than in dogs, even if the worms were from a strain especially adapted to the cat. The environment, i. e., the host, appears to have a more pronounced effect on changing the growth rate of this animal than do any inherent genetic qualities.

Cestodes of the White-Tailed Prairie Dog, Cynomys leucurus. John W. Scott, University of Wyoming.

One adult and two larval species have been found. The adults, *Hymenolepis,* species new, measure from 60 to 250 millimeters, depending somewhat on the degree of contraction of the worms. The scolex has rudimentary rostellum, the neck is short, and the strobila consists of from 430 to 1,005 proglottides in the worms examined. The segments are much broader than long, and the three testes are arranged in a transverse row, one poral and two antiporal to the ovary. The worms occur in about 10% of the host animals, but the distribution in prairie dog towns is rather irregular. The two larval forms are found in the liver; the scolex of each is armed with hooks. One larval species is occasionally found in the spleen, and rarely in the abdominal cavity attached to the mesenteries. As a rule, there is only one individual in each cyst, though many cysts may be clumped close together. It has failed to develop in pups and kittens. The other larval species has been found abundant in only one prairie dog and present in five others. Reproduction by a sort of budding and binary fission occurs in the cysts, and a cyst may contain from one to several specimens of various sizes. Leuckart, 1863, has described a larval Taenia from *Cynomys socialis* which may be identical with one of these.

Use of the Egg Isolation Technic in Epidemiological Studies on Ascariasis in Virginia. L. A. Spindler, Johns Hopkins University.

The technic of egg isolation from soil outlined by Caldwell and Caldwell (1928), consists in freeing the ova with antiformin, in floating them with concentrated sugar solution and in looping from the surface to a slide for examination. Brown modifying the method used sodium bichromate, specific gravity 1.35, in place of sugar solution. In the studies in Virginia, soil was treated with 30% antiformin, then sodium bichromate was added to float the ova which were looped to a slide and examined for developmental stages. This method used in the epidemiological studies on ascariasis in Virginia gave valuable information on the sources of infection especially when no evidence of soil pollution could be found. In one area, soil pollution was absent but 28 soil isolations showed many ascaris eggs present, 68% of which were embryonated, 18% developing, 4% undeveloped and 10% dead, indicating pollution of long duration. Samples from beneath a porch, the children's playhouse, yielded many ascaris eggs, 100% of which were embryonated, showing that no recent pollution had occurred and indicating this spot as the probable source of the children's infections. Sweepings from a kitchen floor yielded 14% undeveloped, 39% developing and 14% dead ascaris eggs showing some development at least under these conditions. During the summer 51 soil isolations from yards of families infested with ascaris were made, 46 being from places where no trace of human stools could be seen. Of this number 48 were positive for ascaris ova showing the efficiency of the method and its value in such studies.

The Occurrence of Self-Cure and Protection in Typical Nematode Parasitism. Norman R. Stoll, Rockefeller Institute. (Invited Paper.)

In a study of the common stomach worm, *Haemonchus contortus,* in sheep, there have intervened self-cure and protection on the part of infected hosts. These phenomena have been elicited under conditions of reinfection, either in the field or with controlled doses of infective larvae indoors, and their degree appears to be correlated with the rate of acquisition of the worm burden. Results show that sheep parasitized by *Haemonchus* respond in effect similarly to hosts parasitized by other kinds of pathogenic microorganisms, i. e., infections may be (1) low grade and perhaps chronic, (2) fulminating and lethal, or (3) self-curative and accompanied by a high and apparently enduring protection against further parasitism by the same worm species. Excluding immunities ascribed to abnormal hosts or to age, the assumption is a familiar one that (depending perhaps primarily on their numbers), worms either tend to kill a host or persist rather indefinitely and more or

less innocuously for both host and parasites. The findings for *Haemonchus,* when considered with other recorded observations, suggest that the phenomena of self-cure and protection are likewise typical reactions to helminthic parasitism, and may be demonstrated by having suitable hosts of known parasitic history secure appropriate doses of worms. With many nematode species located as adults in the gastro-intestinal tract, the means are available of following the degree of parasitism which is attained, independently of clinical effects on the host. These experimental data have a definite bearing on the concept of infection in helminthology.

The Life History of Cryptocotyle lingua (Creplin). H. W. Stunkard, New York University.

Ryder (1884) and Linton (1900) reported metacercariae, encysted in the skins, fins, and gills of *Tautogolabrus adspersus* and other marine fishes from the North Atlantic coast. Linton (1914) showed that these encysted larvae comprise one stage in the life cycle of *Cryptocotyle lingua.* According to Ciurea (1924), the American species of *Cryptocotyle,* which he named *C. americana,* is not identical with *C. lingua* of Europe. During the summer of 1928 the parthenogenetic stages of this species were identified and the life history demonstrated by obtaining development of the larvae in fish and in the cat, rat, and guinea pig. The cercariae occur at Woods Hole in the digestive gland of *Littorina littorea.* They are produced in elongate, sac-shaped rediae which have the flame cell formula $2 \times (3 + 3)$. Liberated cercaria attacked *T. adspersus,* boring into the skin in enormous numbers. The encysted specimens were fed to experimental animals and developed into adults, identical with those found in natural infestations. The cercaria was found by Lebour in the liver of *L. littorea* and *Paludestrina stagnalis* on the British coast. Since *L. littorea* is a European snail, introduced on the American shore during the past century, and since it harbors the same cercaria on both sides of the Atlantic, presumably the species of Cryptocotyle found in America is identical with *C. lingua.* Lebour (1912) tentatively assigned the cercaria from *L. littorea* to *Cercaria lophocerca* Filippi 1857, but the description of that species is too brief to permit a final determination.

Methods for Isolating and Differentiating Species of Eimeria *Occurring in Gallinaceous Birds.* Ernest Edward Tyzzer, Harvard University. (Invited Paper.)

The validity of the name *Eimeria avium* is discussed and evidence for the occurrence of multiple species is furnished. Certain principles applying to the Eimeria infections studied may be reviewed before passing to the discussion of methods, i. e., cyclical development, dosage and immunity. With the recognition of the occurrence of multiple species, two distinct but somewhat related problems arise, i. e., one the isolation, the other the differentiation of species. It is occasionally possible to obtain, adventitiously, material containing only a single species, from its occurrence alone in a given host. Advantage may be taken of the distribution of a parasite so that the selection of material from some part of the intestine may furnish a single species. In some species which develop more rapidly than others, the collection of the first oocysts to be discharged in an experimental infection may be sufficient for isolation. Under conditions of continuous reinfection certain species disappear more promptly than others on account of the development of immunity of the host, and under such circumstances the late collection of material may yield a single species. "Pure lines" may be obtained through the isolation of a single oocyst, which may be accomplished in some instances by the dilution method. This method is not practicable when the organism sought is poorly represented in the mixture. Under such circumstances the organism may be isolated by the Barber pipette. In the recognition of species, it appears to be a safer course to compare various features than to depend on one alone. The characteristics that may be taken into account are the following: 1. The length of the period of development up to the discharge of oocysts. 2. The time required for sporulation at a given temperature. 3. Host specificity or degree of restriction to a

given host. 4. Characteristic habitat or distribution of the organism in its host. 5. Cross immunity tests. 6. Pathogenicity as determined by both experimental and natural infection. 7. Morphological studies, applying as well to the forms developing in the tissue and not confined solely to the size and shape of the oocyst. 8. The relation of parasite to host-cell, together with the reaction of the latter. The recognition of various types of coccidiosis in poultry is often possible from the gross pathological changes, but, for the accurate determination of species, it is essential to take into account various characteristics such as are here discussed.

Some Biological Aspects of the Acanthocephala. H. J. Van Cleave, University of Illinois. (Invited Paper.)

Analytical and historical reviews of the development of morphology and taxonomy of the Acanthocephala are given. Morphological characters utilized in specific, generic, and super-generic categories are discussed and evaluated in the light of recent advance. The life cycle hosts, and effect upon the host are presented in outline. The distribution of the Acanthocephala with especial reference to the distribution of the hosts and to geographical distribution are discussed.

A Human Skin Disease Due to Larvae of Ancylostoma caninum. G. F. White and W. E. Dove, U. S. Department of Agriculture.

During experimental studies on creeping eruption two types of lesions were produced when larvae from cultures containing both *Ancylostoma braziliense* and *A. caninum* were applied to the human skin. These are (a) papules followed by the linear lesions that characterize creeping eruption, and (b) papules unaccompanied by such lesions. Using pure cultures lesions of the former type were shown to be due to *A. braziliense,* while those of the latter were produced by *A. caninum.* To what extent papules without linear lesions may result from braziliense infections and papules with them from caninum ones is yet to be determined. During the first day following invasion of the skin by *A. caninum* there is itching, urticaria-like elevations about the points of ingress of the larvae, erythema and beginning papule formation. During the second day the papules become well formed and much itching is present. During the third and fourth days the reaction begins to subside. By the end of the first week much improvement is noted and by the end of the second week recovery is almost complete. Further studies are needed for conclusive proof that a dermatitis due to *A. caninum* is a clinical entity as well as an experimental one. The observations made thus far indicate strongly, however, that it is. A skin disease due to larvae of *A. caninum* is naturally one of much interest in itself. Since it is somewhat similar to and probably occurs occasionally as a mixed infection with creeping eruption it has an added importance in the diagnosis and treatment of the later disease.

Demonstration of Inexpensive Apparatus for Making Motion Pictures of All Stages in the Life History of Parasites. A. E. Woodhead, University of Michigan.

A demonstration is given of a laboratory devised apparatus constructed to take motion pictures in all degrees of magnification with a minimum amount of adjustment, also to make photographs up to lantern slide size. It is capable also of being used as a projector.

The Life History of a Gasterostome, Bucephalus, Demonstrated by Motion Pictures. A. E. Woodhead, University of Michigan.

The film clearly demonstrates the value of micro-motion pictures to the parasitologist. All stages in the life history of the parasite are shown as well as the method of handling and examining the intermediate and final hosts. Locomotion is especially well brought out as well as certain aspects of behavior of the motile stages.

BOOK REVIEW

DOCUMENTA MICROBIOLOGICA, MIKROPHOTOGRAPHISCHER ATLAS DER BAKTERIEN, DER PILZE UND DER PROTOZOEN. By JULJAN NOWAK. Theil 1. Jena, Verlag von Gustav Fischer, 1927.

The author has embarked upon an ambitious task and the appearance of the first volume leads one to hope for the early completion of the series. If done with equal merit it will form a most valuable aid for workers in the broad field of parasitology. The original intention of the author was to publish a few microphotographs of pathogenic bacteria in order to give the student in the laboratory some proper conception of form and appearance and to lighten the work of instructors who find it so difficult to indicate in words the characteristic peculiarities of individual organisms. In bringing together materials new morphological characteristics were constantly coming up until the series assumed much larger proportions than originally intended. It also appeared necessary to include the fungi and protozoa as disease producing organisms of first importance. The present volume includes only the bacteria with some algae and a few organisms of uncertain relationship. The photographs are admirable and are reproduced in a way to do great credit to the publisher. Subsequent volumes will be welcomed if they attain measurably the same high standards of excellence manifested in this one.

A recent publication entitled *On Rous, Leucotic and Allied Tumours in the Fowl* by J. P. McGowan (The Macmillan Co., 1928) will be of interest to some parasitologists who are studying the effects on tissue of the intrusion of parasites. Certain features of cell aggregation, the presence of melanin and some other factors noted recall the phenomena encountered in certain cases of parasitic invasion.

A discussion recently published, entitled *Probleme der pathologischen Physiologie im Lichte neuerer immunbiologischer Betrachtung* by Professor Hans Sachs of Heidelberg University (Verlag von Julius Springer, Wien, 1928) touches closely upon some of the questions newly raised in the field of parasitology and will prove interesting reading for students in that field.